
Principles of Biochemistry

Third Edition

International Student Version

Donald Voet • Judith G. Voet • Charlotte W. Pratt



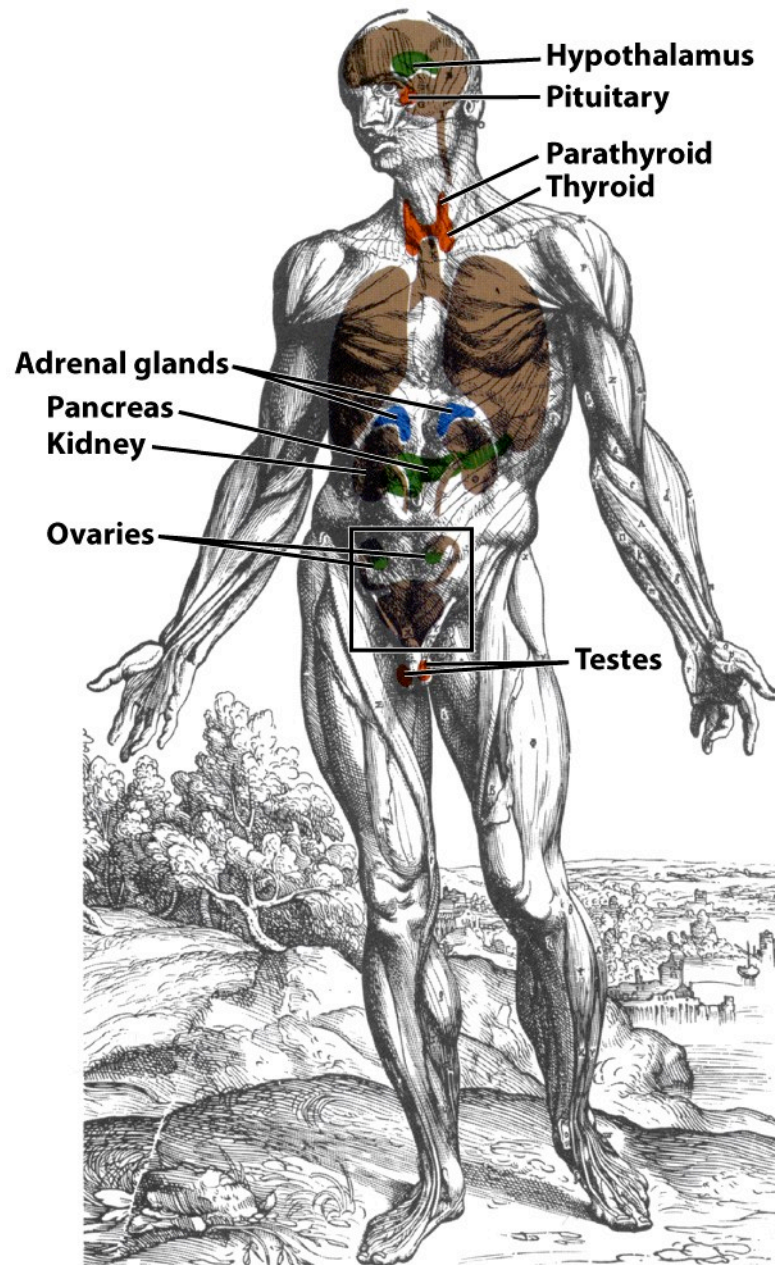
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Chapter 13

Biochemical Signaling

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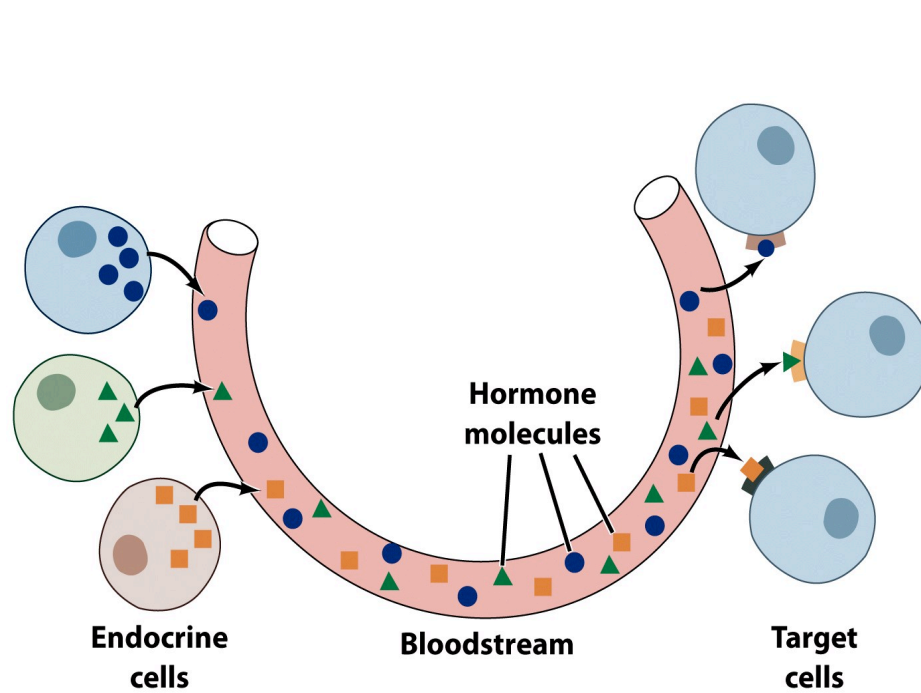
HORMONES



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Figure 13-1

- **Maintenir Homeostase**
- **Répondre à des stimuli externes**
- **Réguler des cycles (maturation, cycle menstruel, differentiation, etc)**



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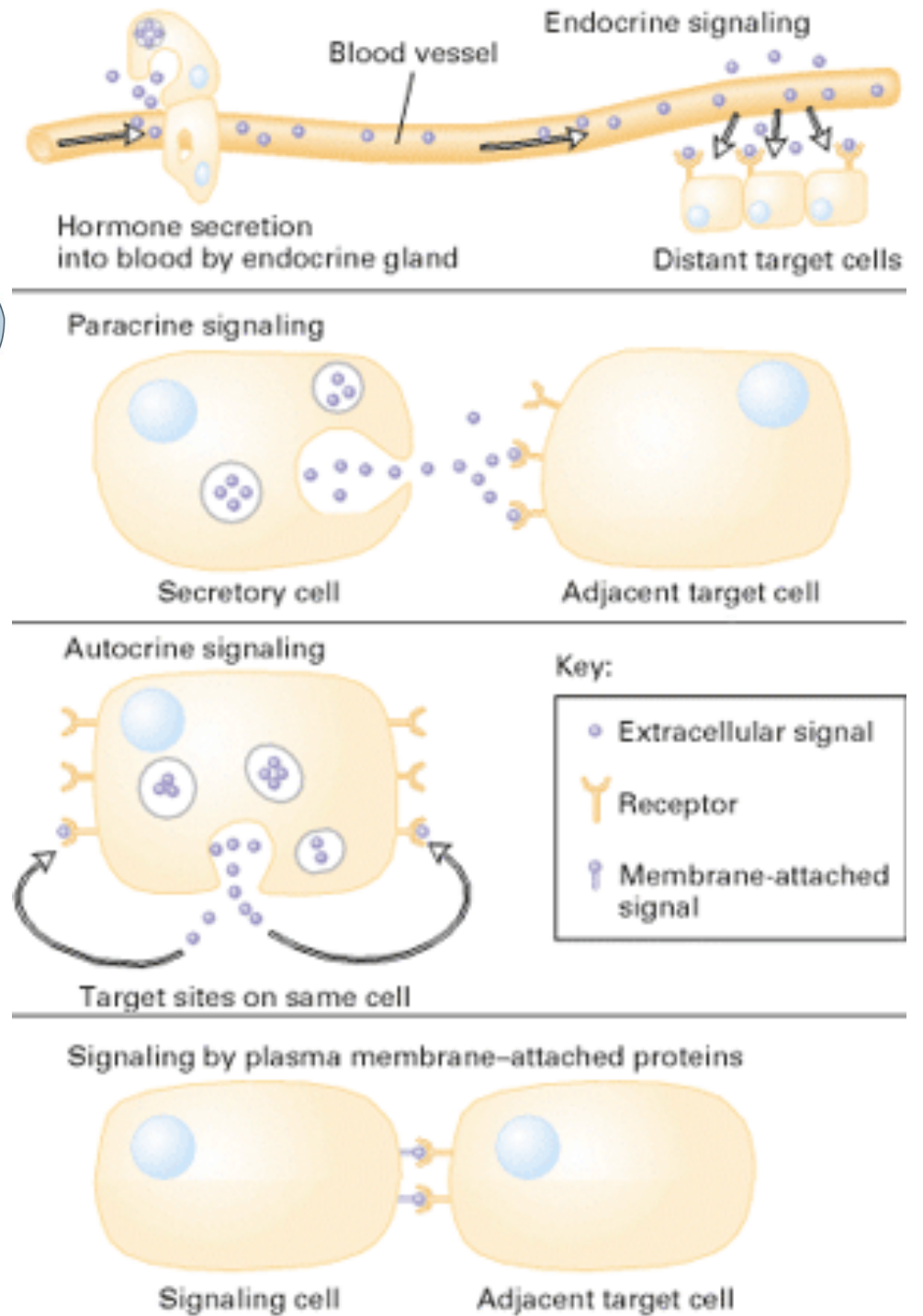
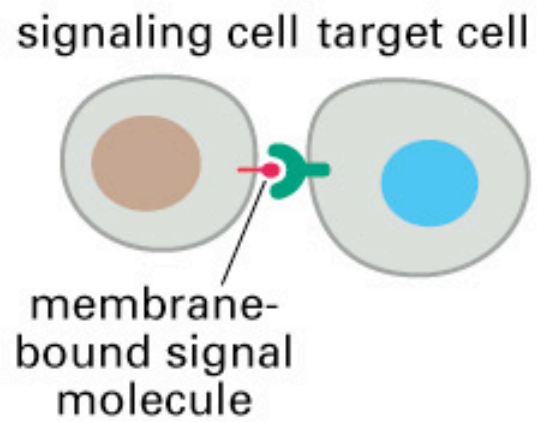
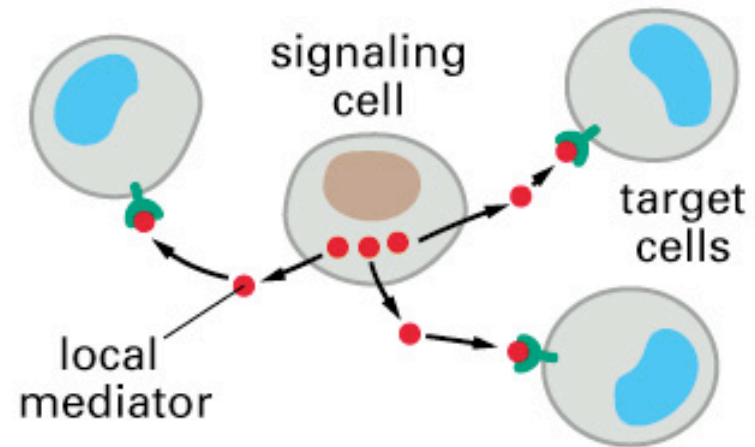


Figure 13-2

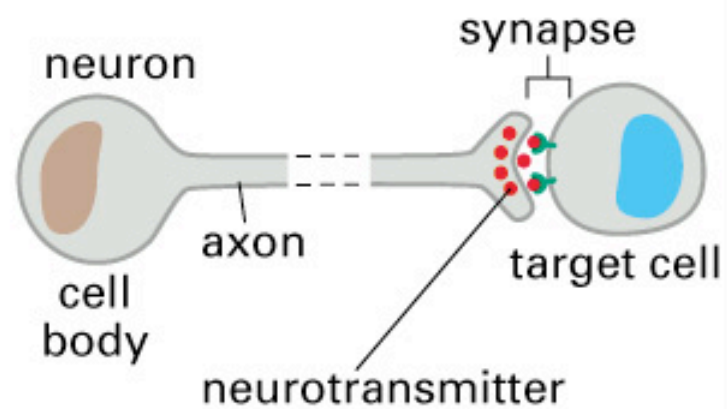
(A) **CONTACT-DEPENDENT**



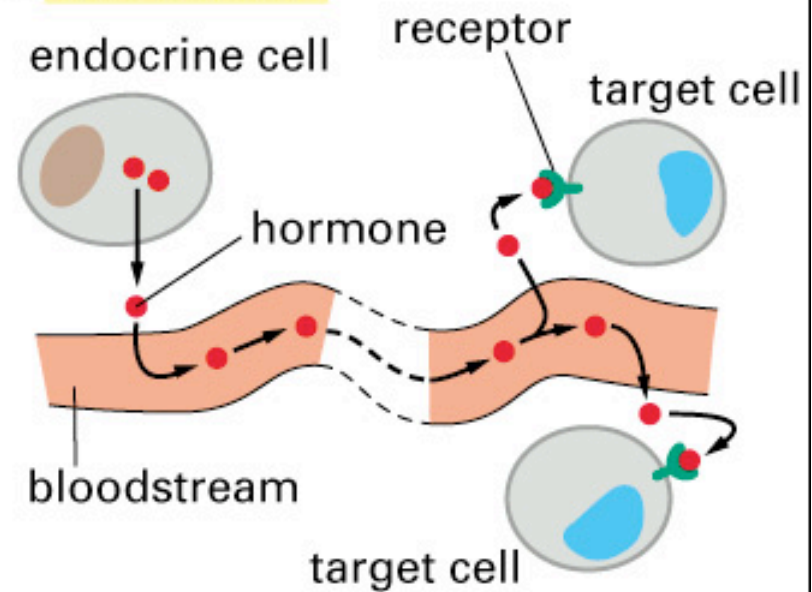
(B) **PARACRINE**



(C) **SYNAPTIC**



(D) **ENDOCRINE**



Receptors

= Proteins that bind signals and initiate a signaling cascade

Cell membrane receptors

-integral membrane proteins that bind an extracellular signal and start a signal cascade

Intracellular receptors

-nuclear hormone receptors

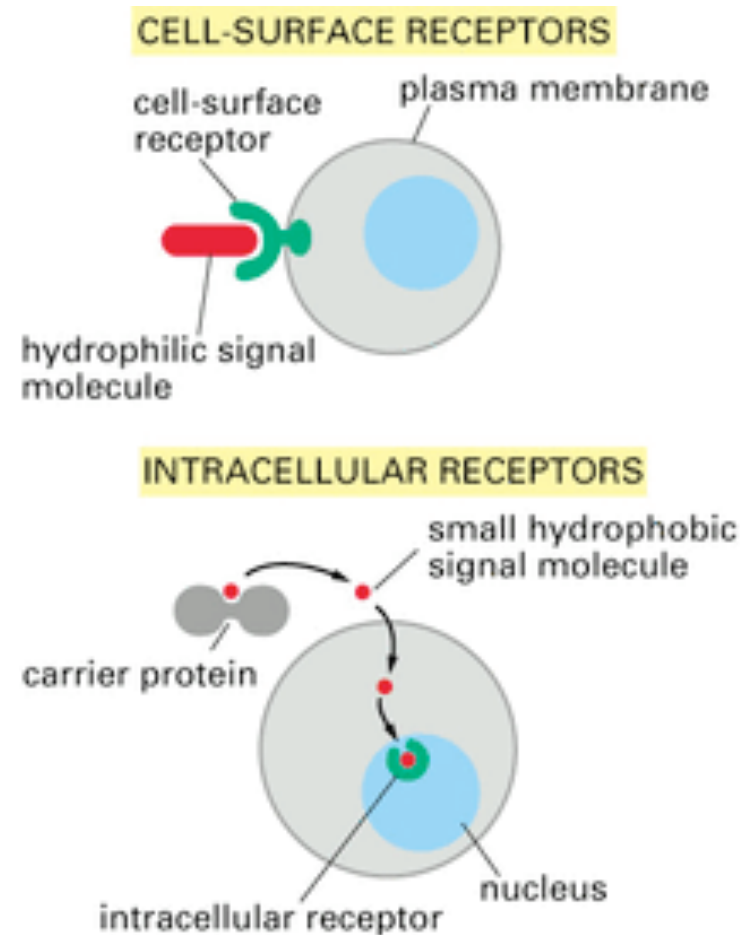


Figure 15-3. Molecular Biology of the Cell, 4th Edition.

Nuclear hormone receptors

Examples include

- steroid hormone receptor and
- thyroid hormone receptor
- Retinoic acid receptor
- Vitamine D receptor

NHRs are **transcription factors** that respond to specific ligands

Ligands alter the ability to bind to specific DNA regulatory elements

Receptor Properties

A hormone binds to a Receptor

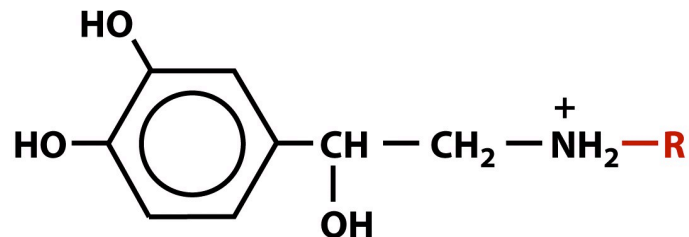
- soluble (cytoplasmic) for lipophilic hormones:
 - * arachidonic acid derivatives (leukotrienes, retinoic acid, prostaglandins)
 - * vitamin D3 and steroids
 - * thyroid hormones (T3 & T4)
- membrane bound (at the plasma membrane): for peptides and neurotransmitters

Often the same Hormone can bind different Receptors

=> different signaling pathways => different responses

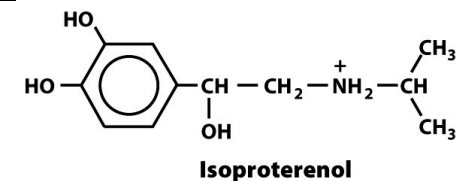
=> receptor subtypes (role of agonists/antagonists)

ex: Adrenaline binds to a (α_1 , α_2 , α_3) or (β_1 , β_2) receptors

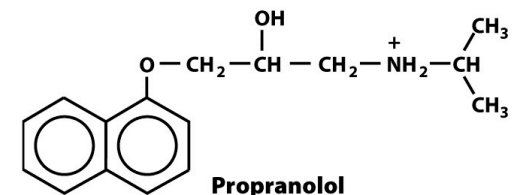


R = H Norepinephrine (noradrenalin)

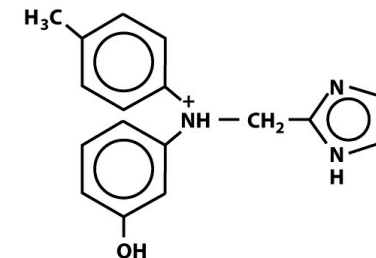
R = CH₃ Epinephrine (adrenalin)



Isoproterenol



Propranolol

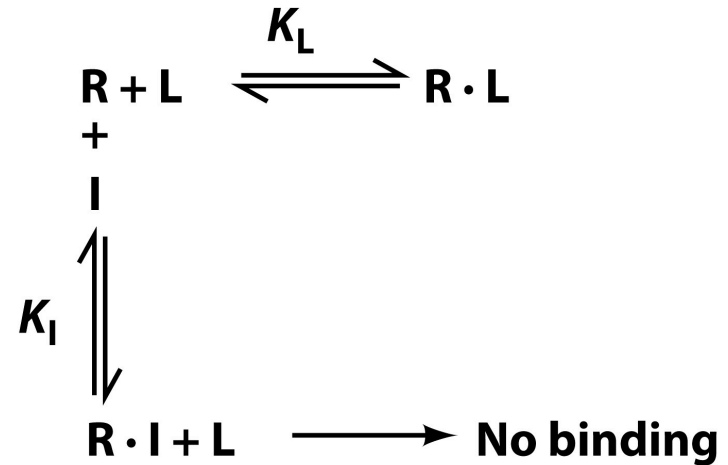


Phentolamine

Liaison Ligand-Recepteur

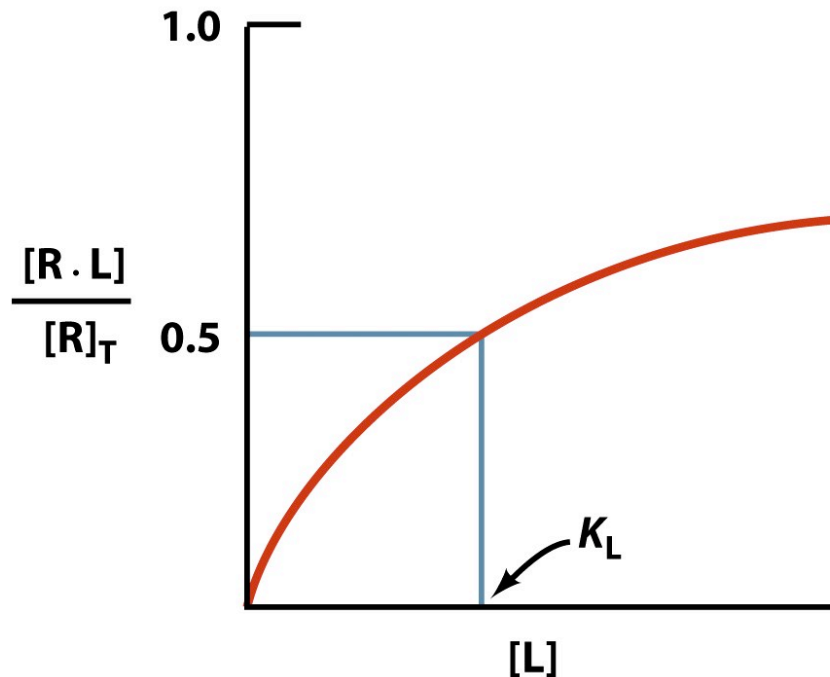


$$K_L = \frac{(R)(L)}{(R \cdot L)} = \frac{((R)_T - (R \cdot L))(L)}{(R \cdot L)}$$

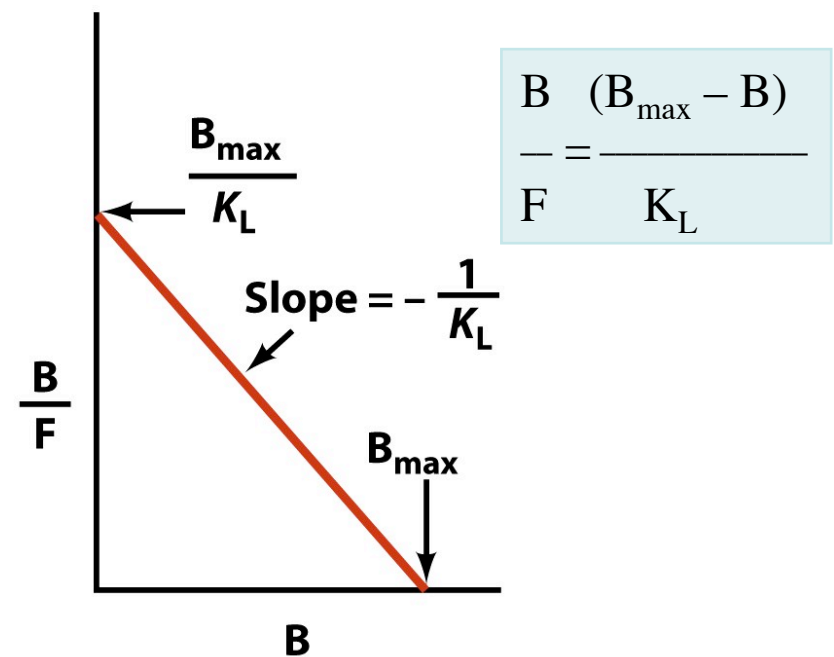


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(a) Diagramme hyperbolique



(b) Diagramme de Scatchard



General Principles of Signal Transduction

1. Communication usually involves
- (i) a signaling molecule,
 - (ii) a receptor,
 - (iii) intracellular signal transducers and
 - (iv) targets

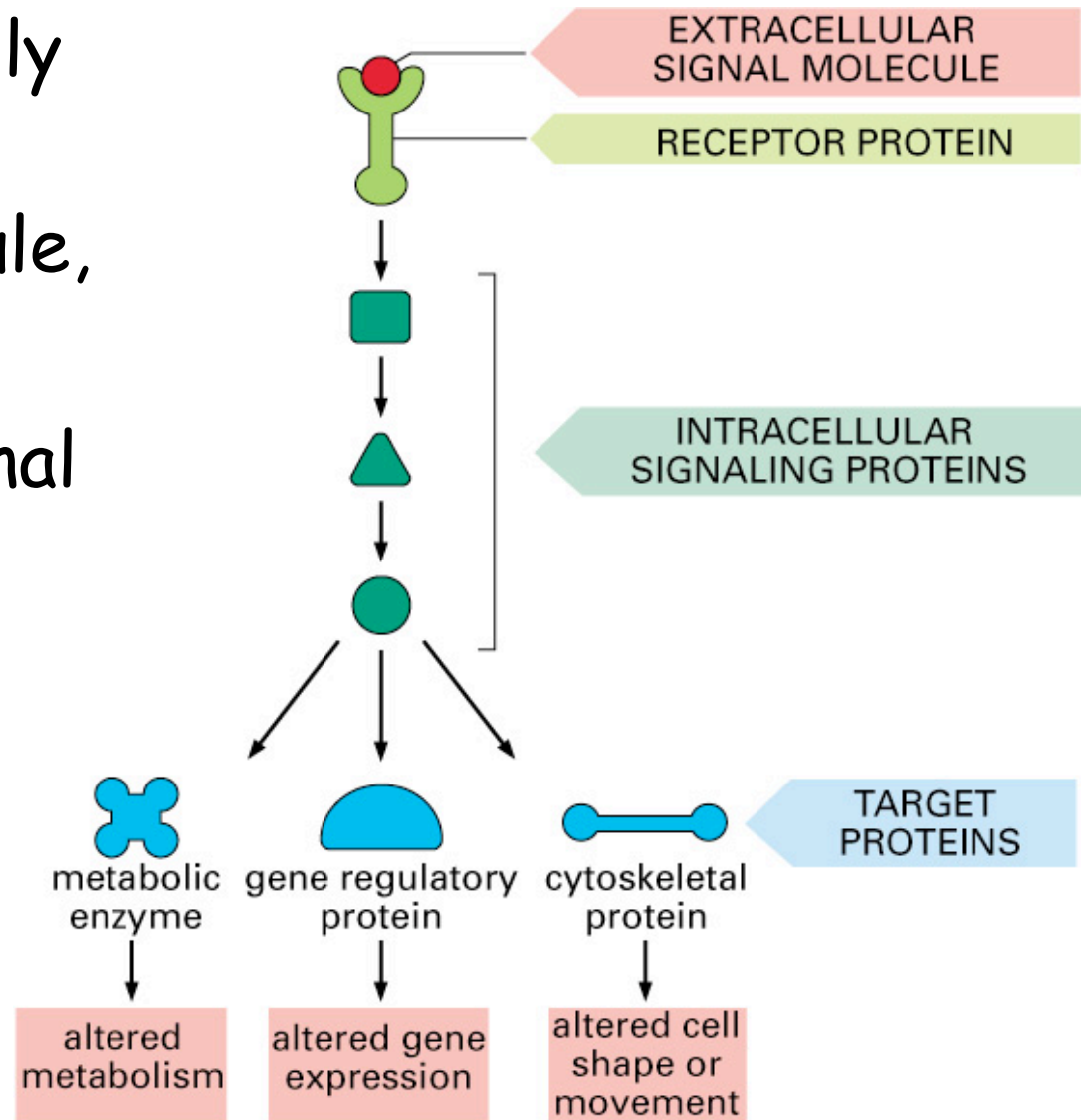


Figure 15-1. Molecular Biology of the Cell, 4th Edition.

General Principles of Signal Transduction

2. Each cell responds to a complex profile of signaling molecules (crosstalk)

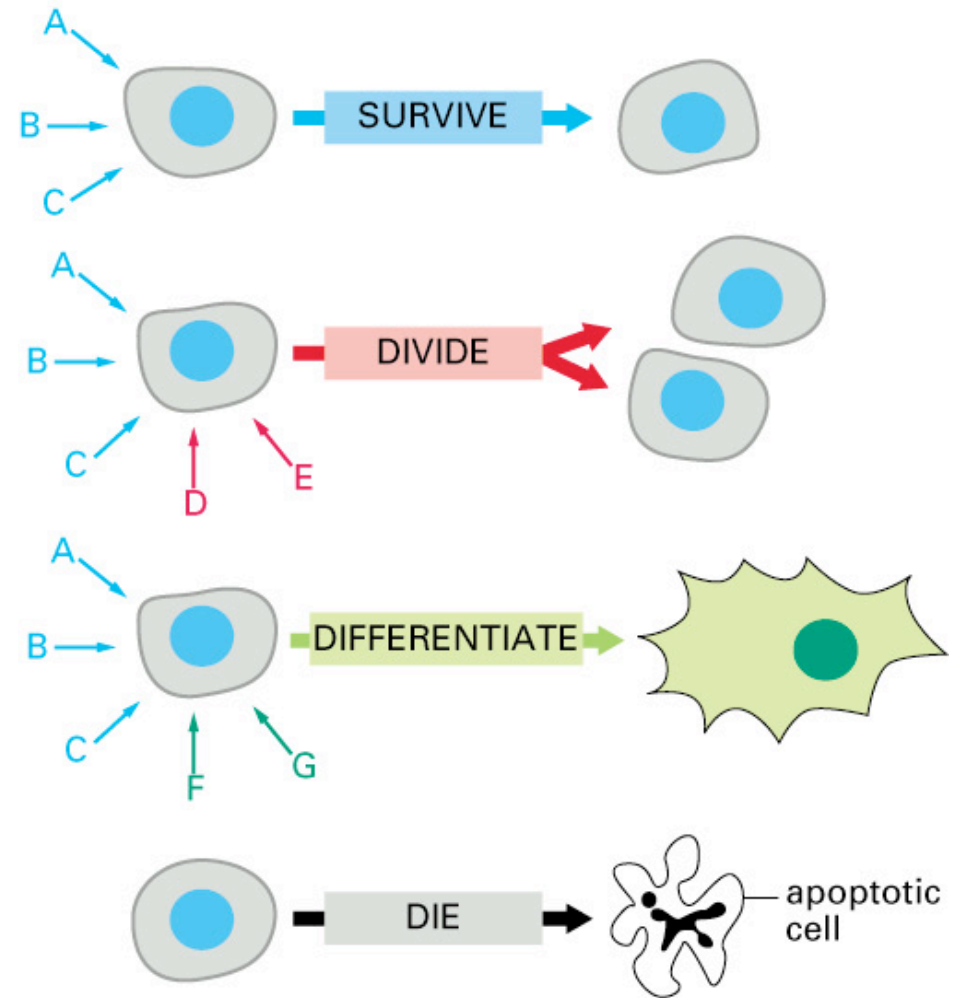


Figure 15–8. Molecular Biology of the Cell, 4th Edition.

General Principles of Signal Transduction

3. Different cells respond differently to a particular signaling molecule

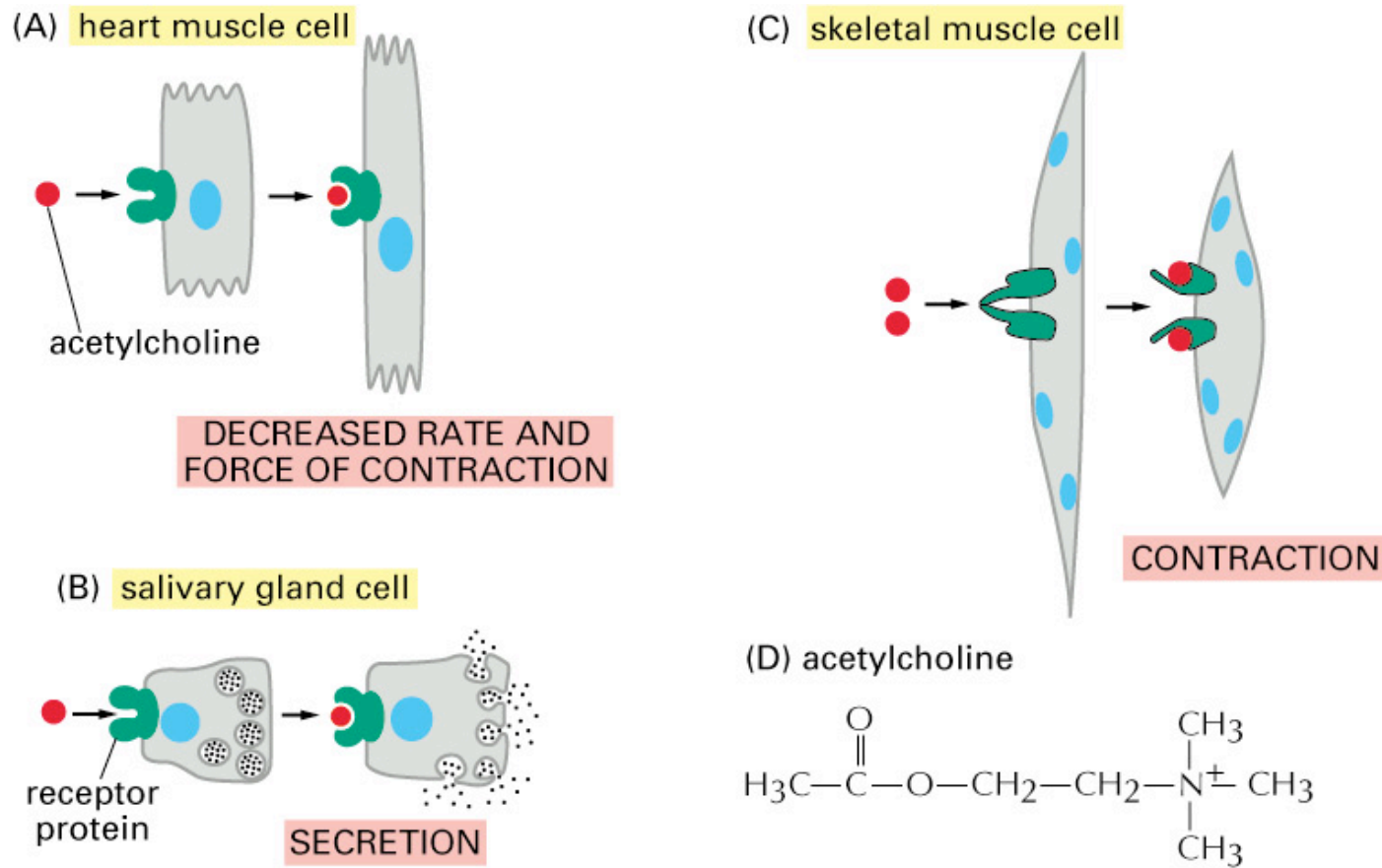
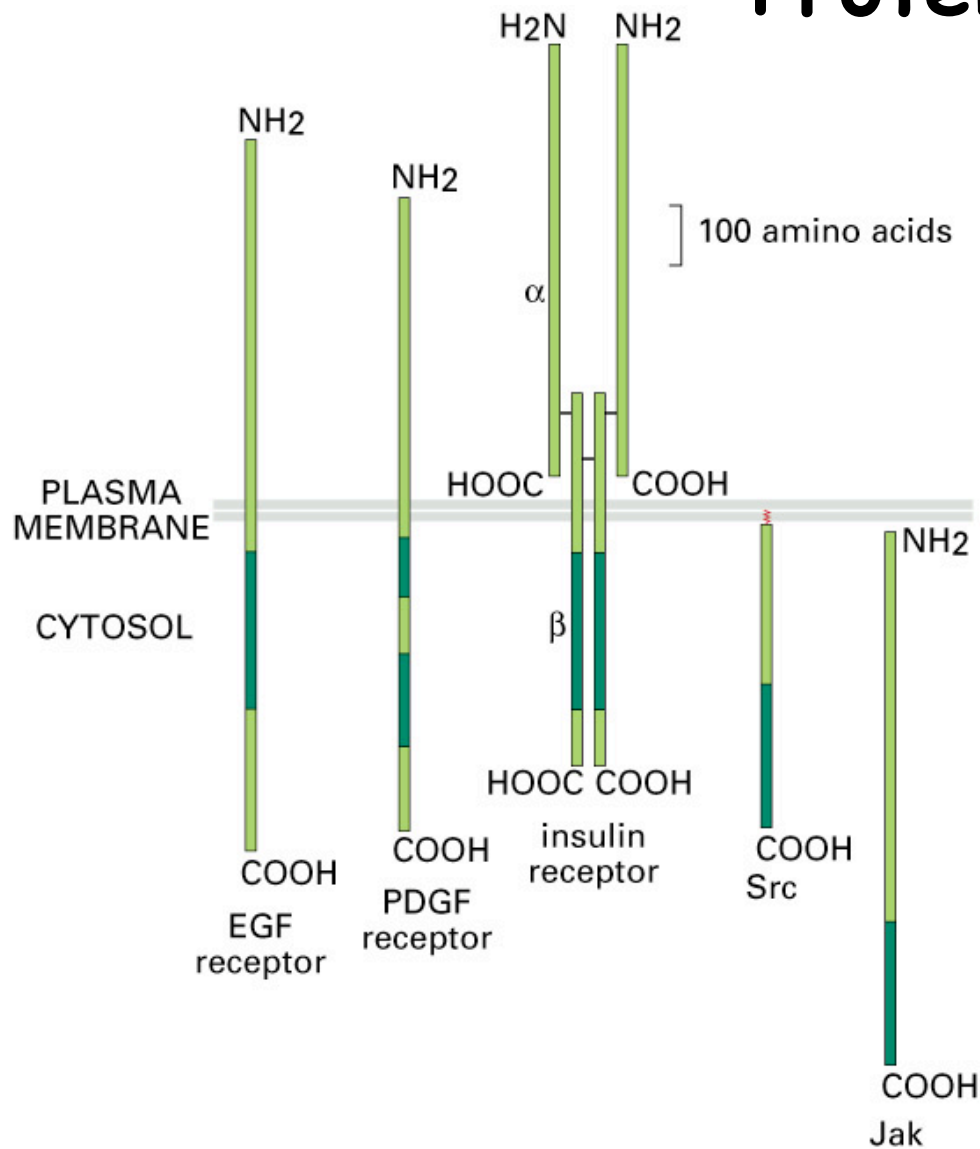
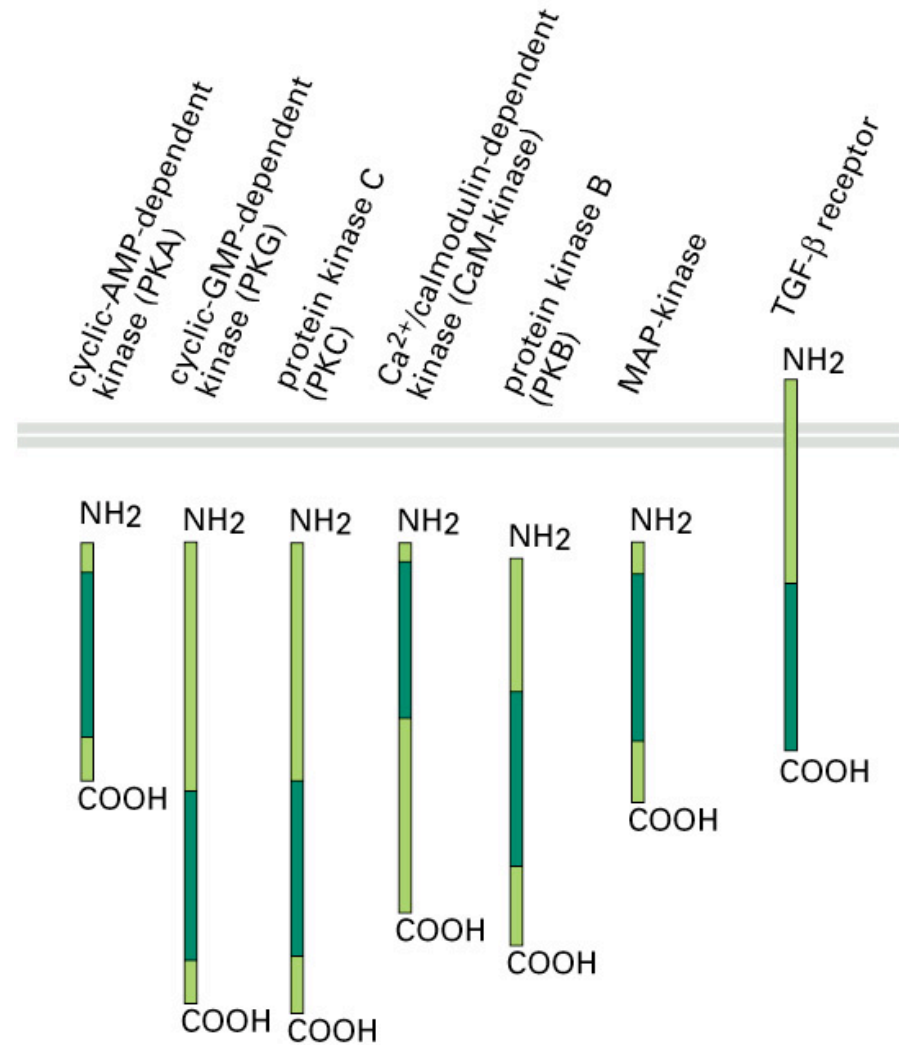


Figure 15-9. Molecular Biology of the Cell, 4th Edition.

Protein kinases



TYROSINE-SPECIFIC PROTEIN KINASES



SERINE/THREONINE-SPECIFIC PROTEIN KINASES

Receptor tyrosine kinases

Most common type of receptor for many common protein hormones including EGF, PDGF, FGF, HGF, IGF-1, VEGF, NGF.

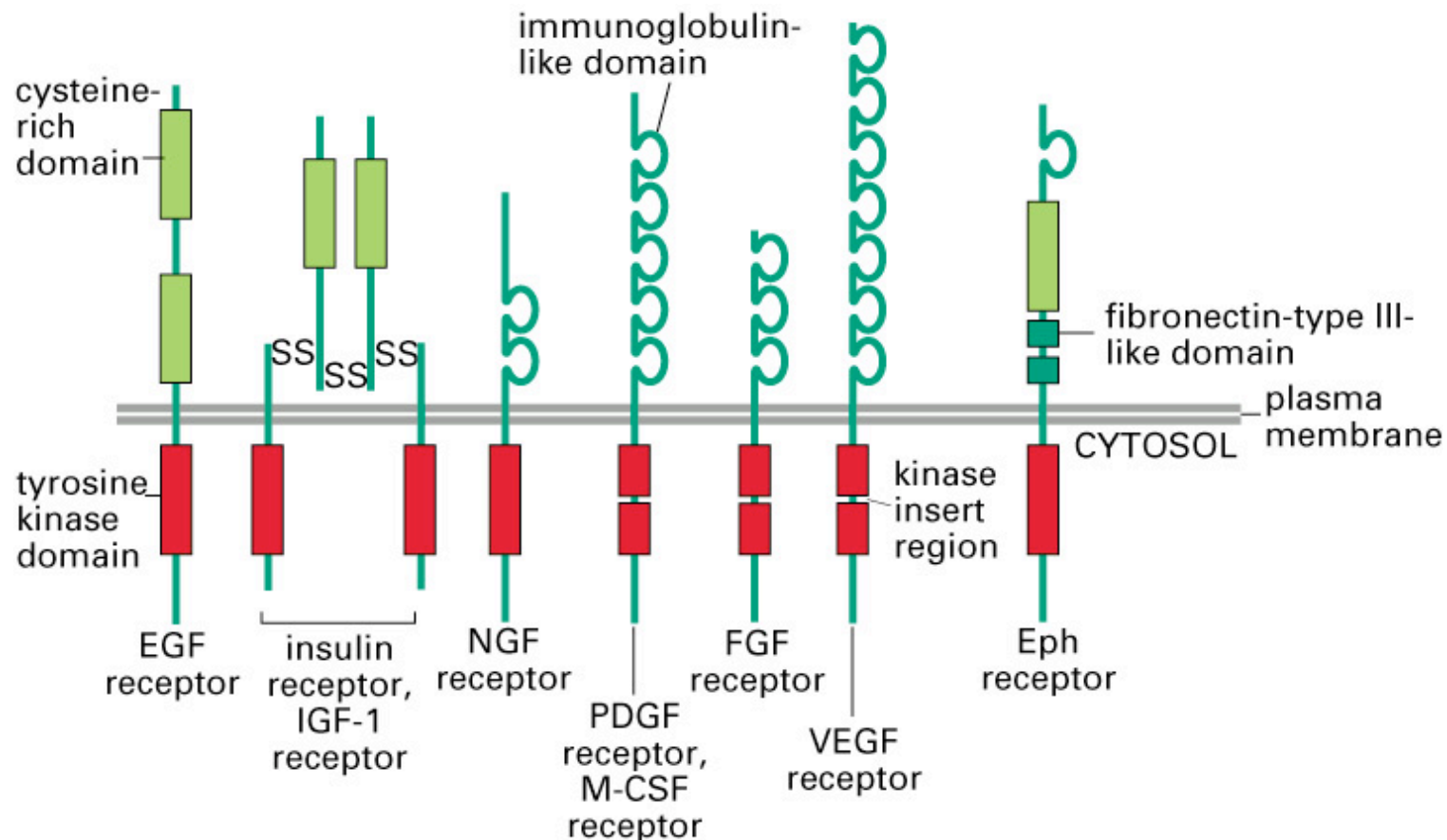
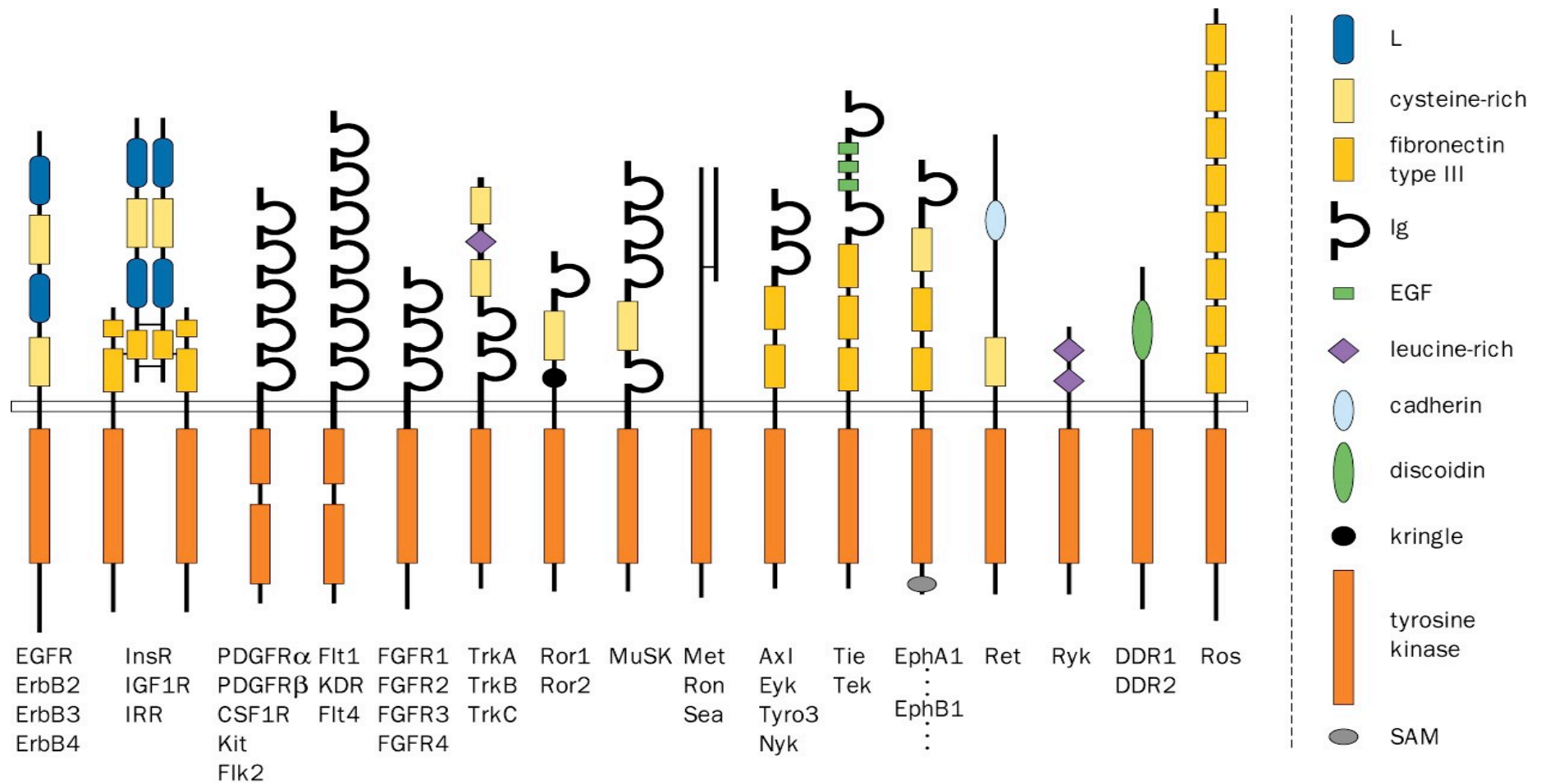


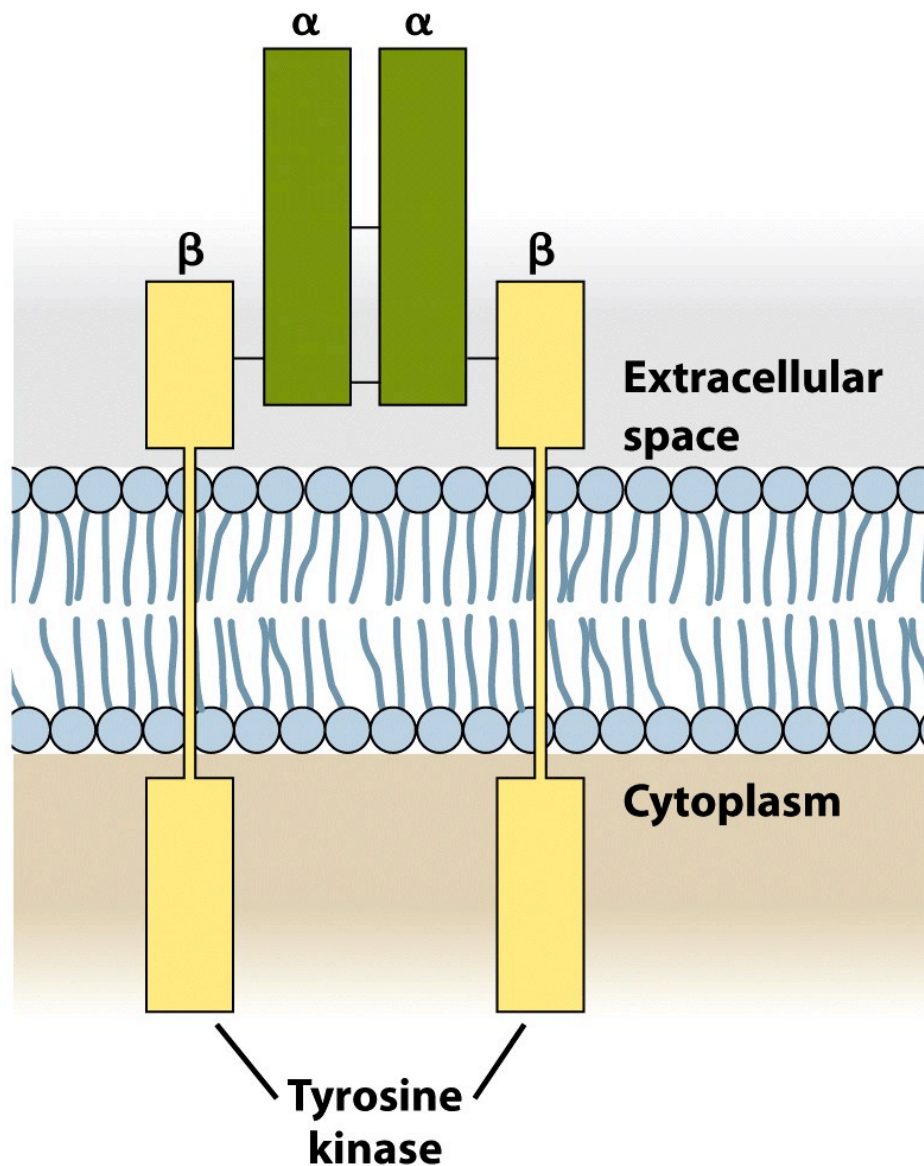
Figure 15-49. Molecular Biology of the Cell, 4th Edition.

Domain organization in a variety of receptor tyrosine kinase (RTK) subfamilies.

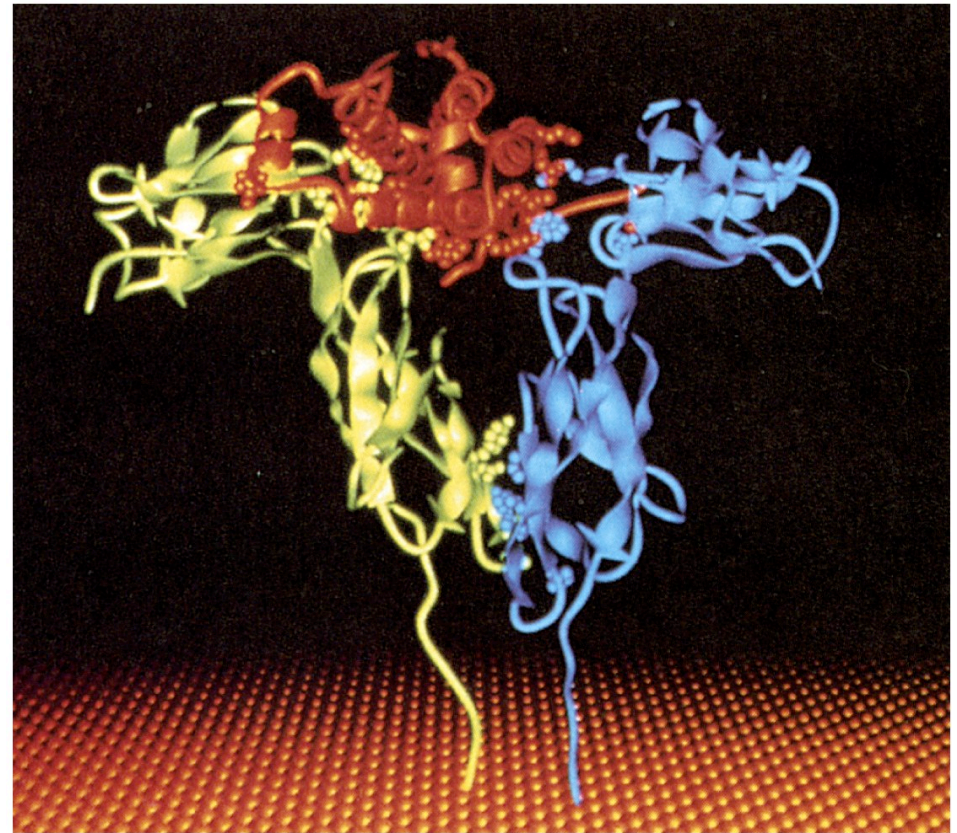


Recepteurs à activité Tyrosine Kinase

Recepteur d'Insuline



Recepteur de hGH (hormone de croissance)



Courtesy of Abraham de Vos and Anthony Kossiakoff Genentech Inc., South San Francisco, California

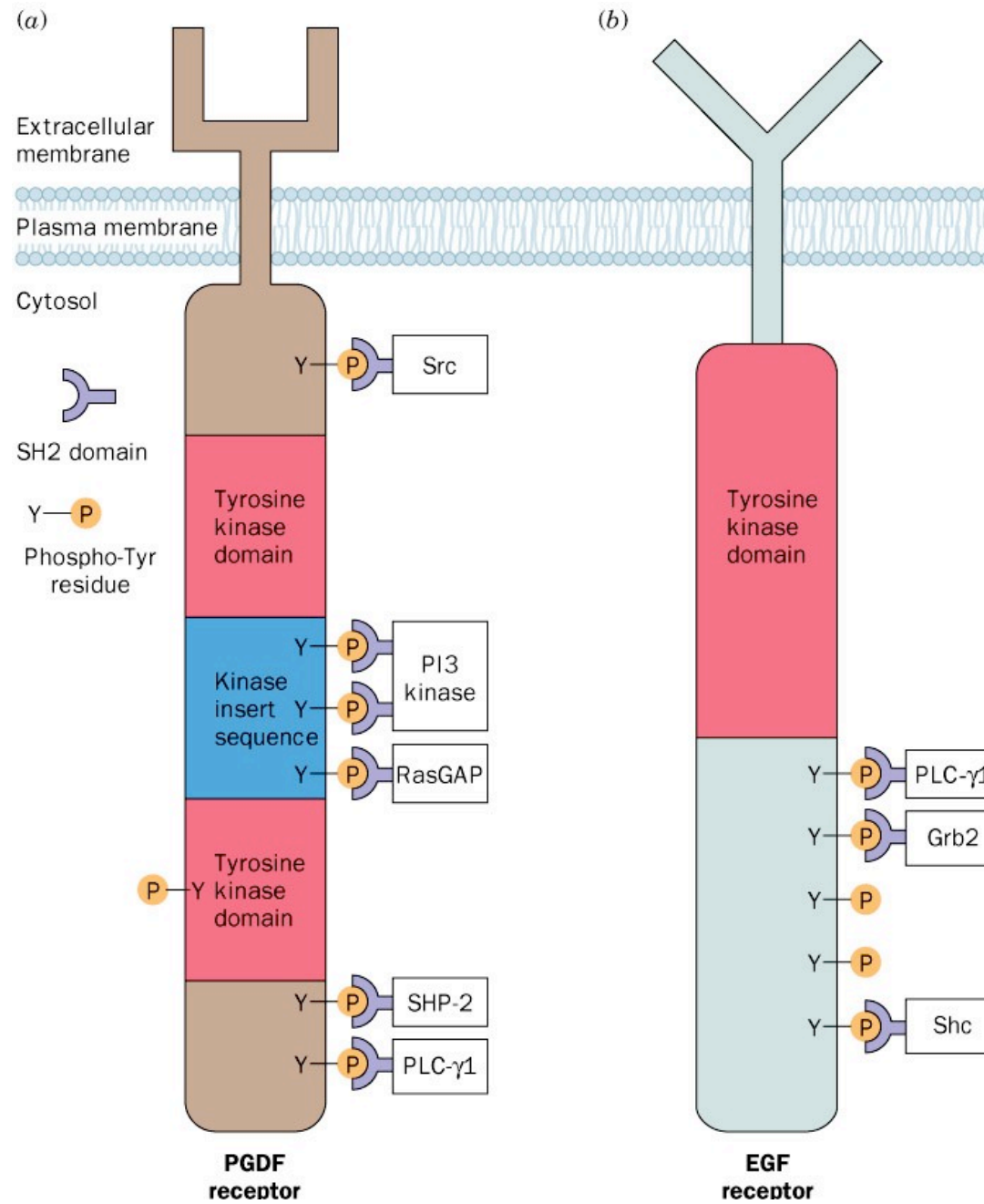
Recepteurs tyrosine kinases

Le Recepteur possede une activité *intrinsèque* tyrosine kinase

Lorsque le ligand se lie, le recepteur se dimerise et développe une activité tyrosine kinase

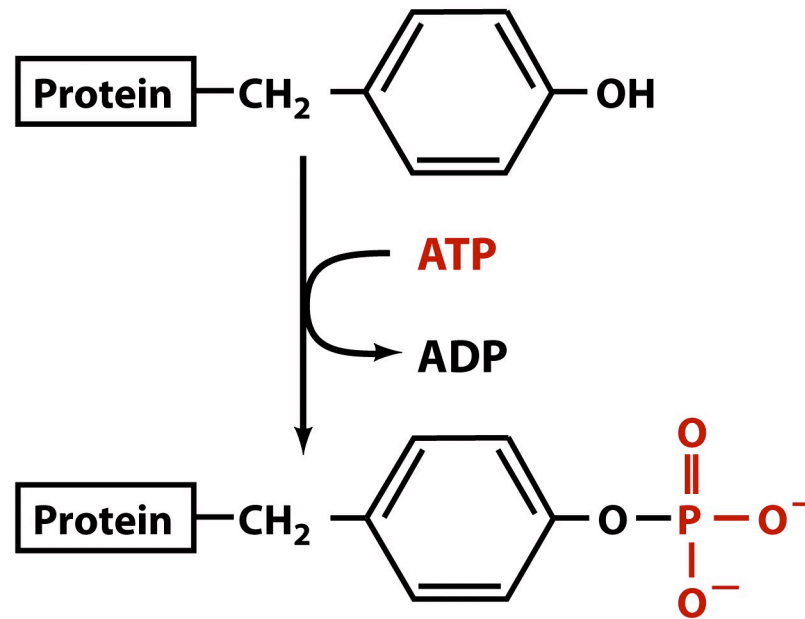
Il s'auto-phosphoryle (autophosphorylation), provoquant:

1. une activité kinase plus forte
 2. une plus forte affinité pour d'autres proteines
- Une fois liées ces protéines seront phosphorylées

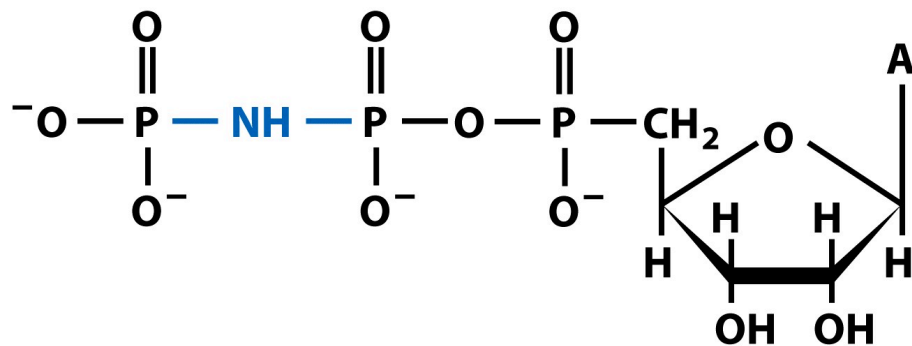


Schematic diagrams of RTKs.

1. Recepteurs à activité Tyrosine Kinase



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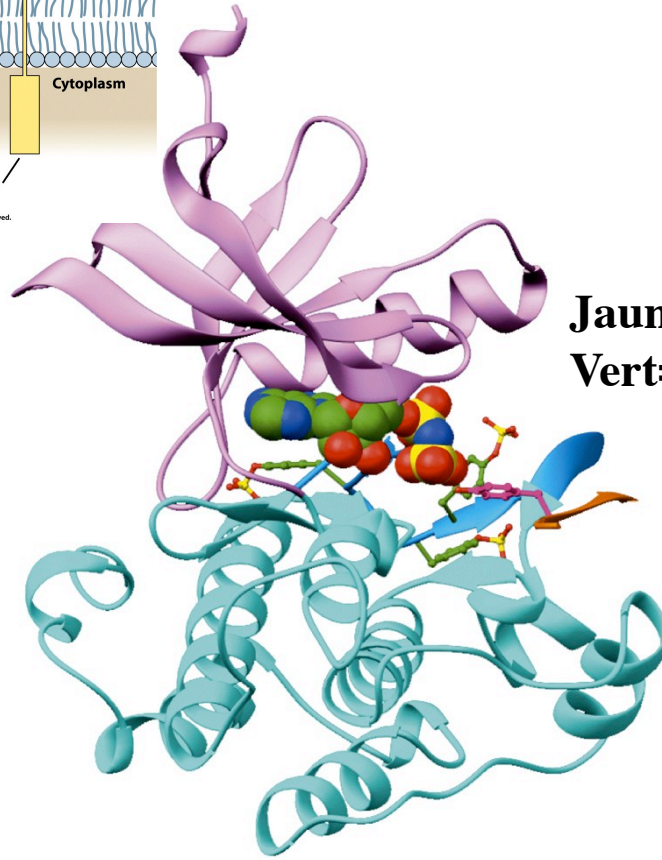
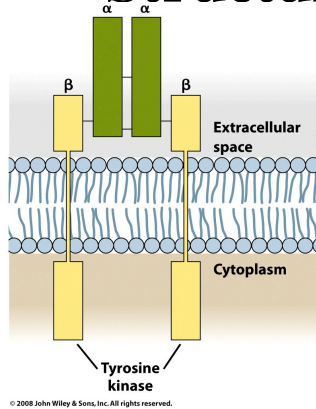


Analogue d'ATP

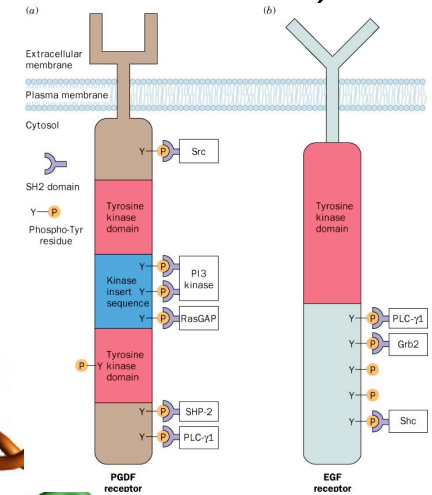
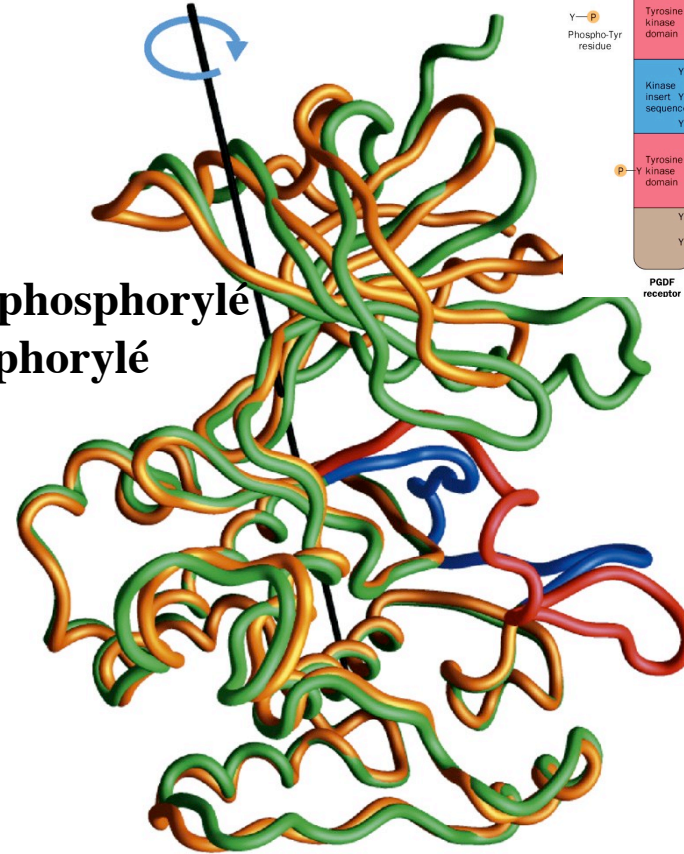
**Adenosine-5'-(β,γ-imido)triphosphate
(AMPPNP)**

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Structure du Domaine Tyrosine Kinase (Recepteur d'Insuline)



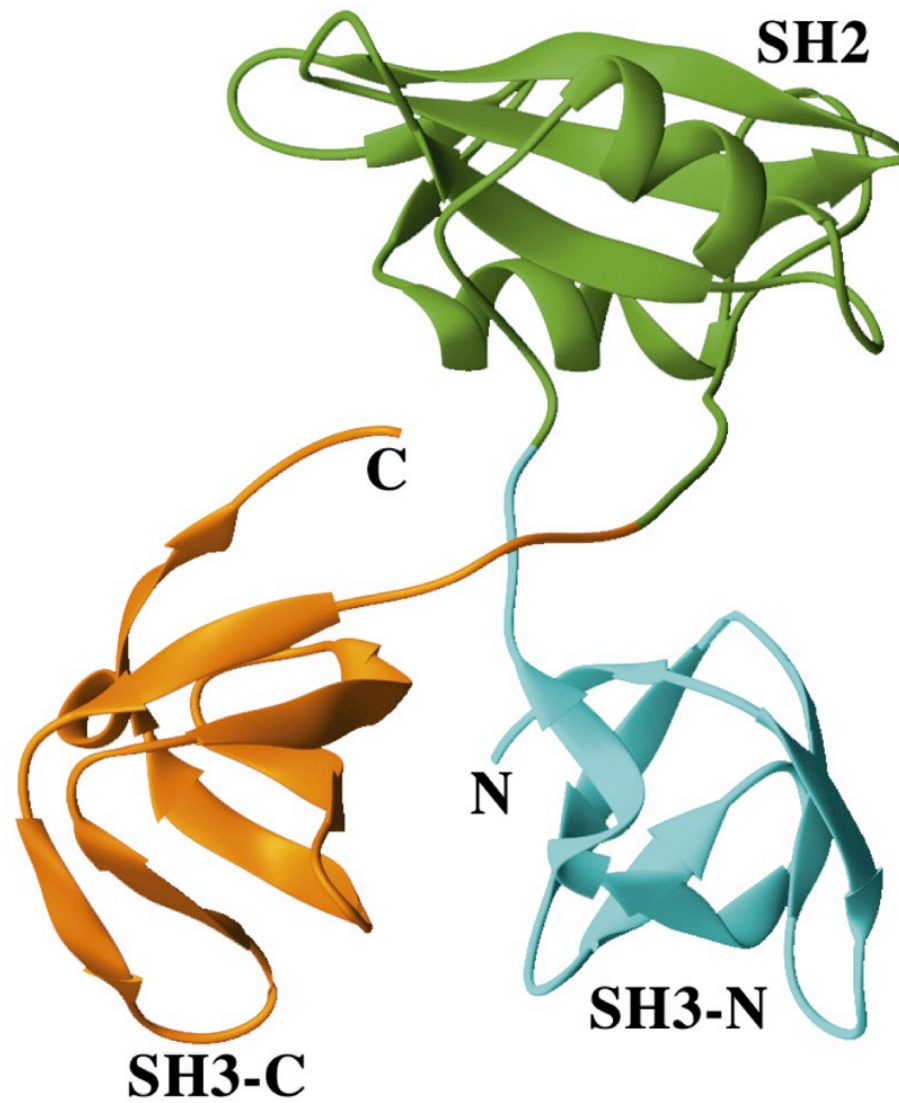
Jaune=déphosphorylé
Vert=phosphorylé



PTK Domain

undergoes major conformation change &
autophosphorylation (1 to 3 Tyr residues)

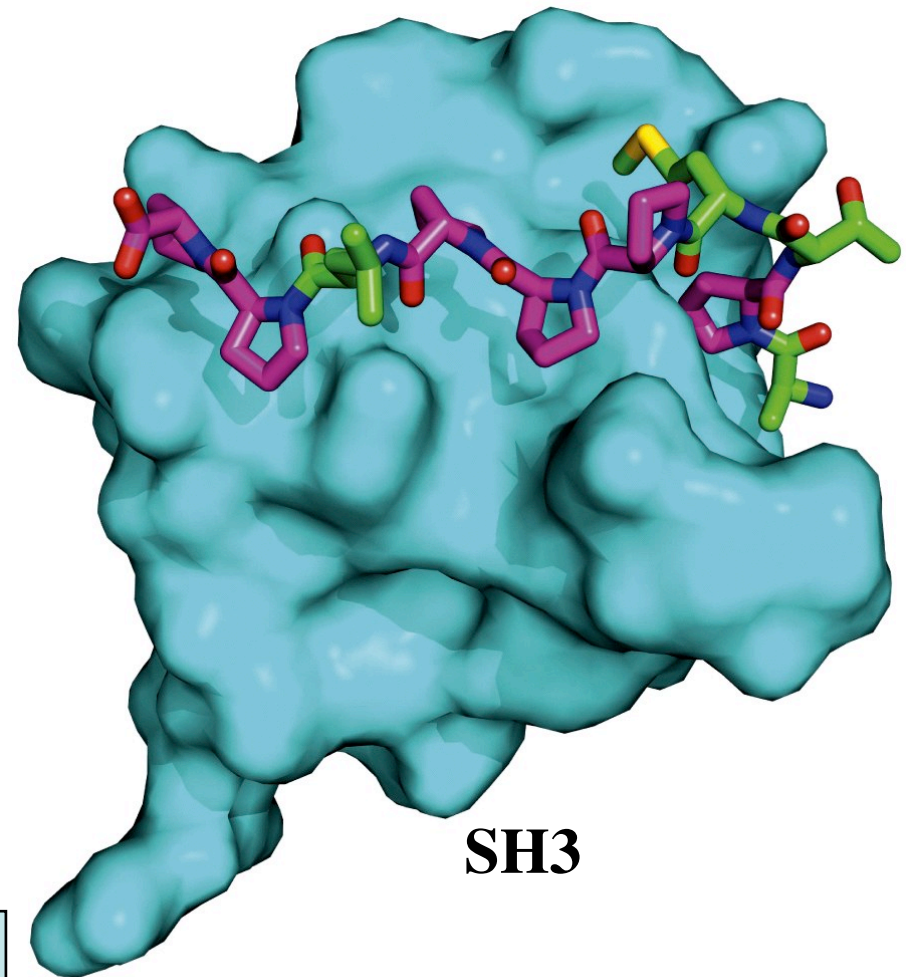
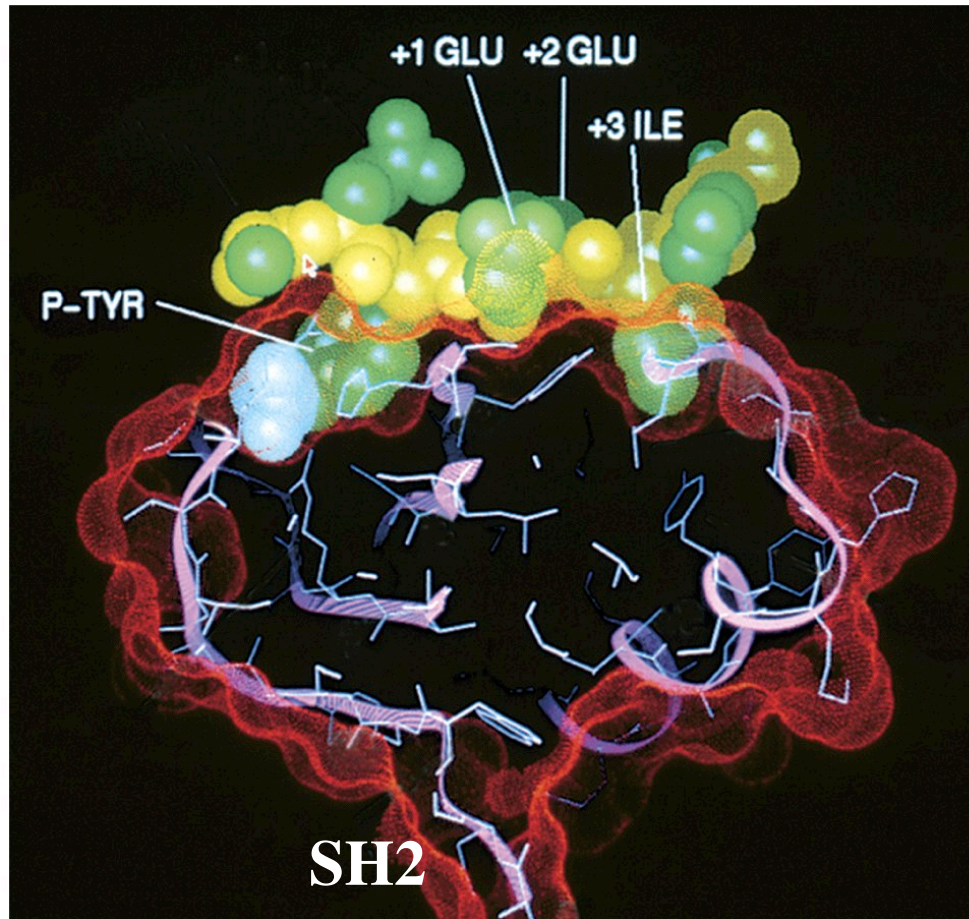
Structure des Domaines SH2 & SH3 (de Grb2)



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Figure 13-9

Structure des Domaines SH2 & SH3 (Recepteur d'Insuline)

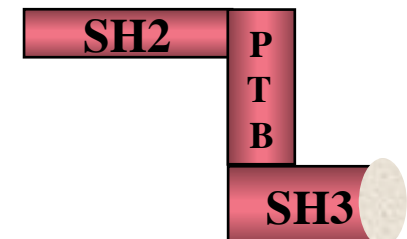


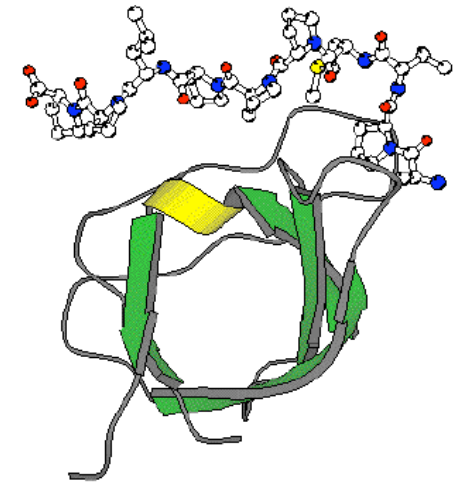
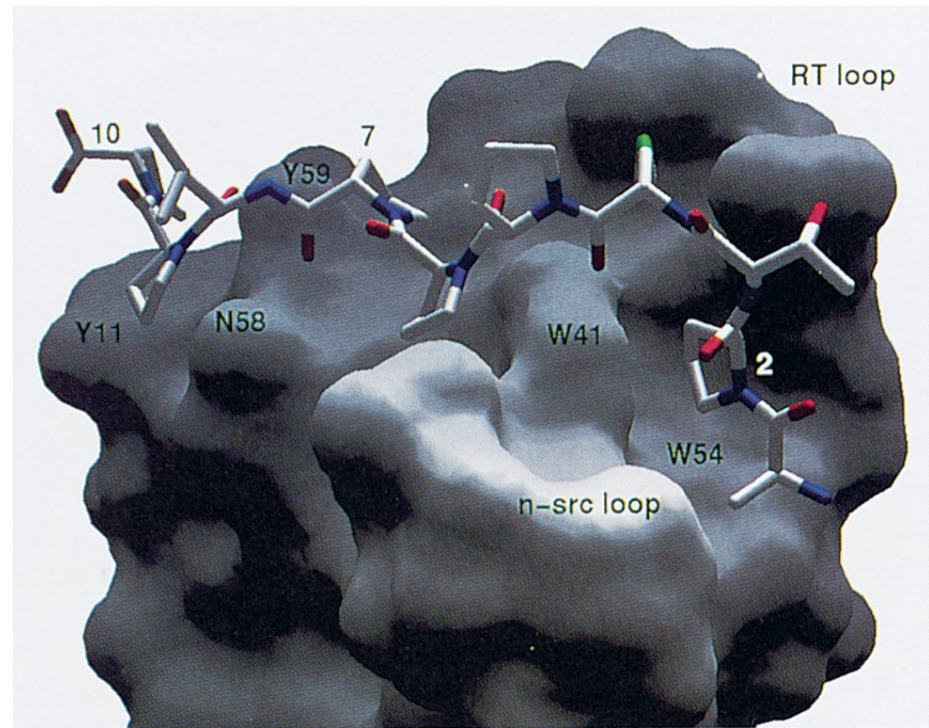
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Relaying the signal:

Binding Modules, Adaptors, GEF, GAP

- SH2 domains mediate signal Transduction
- PTB domains bind pY-containing peptides
- SH3 domains bind Pro-rich peptides





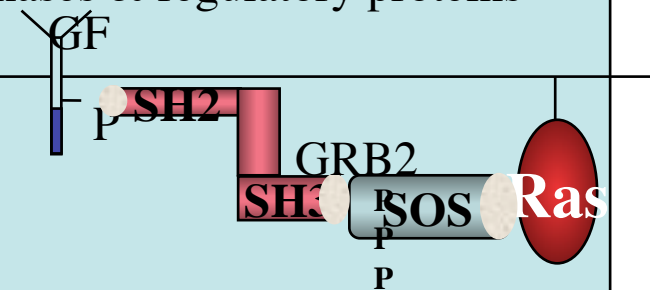
Structure du domaine SH3 de Abl dans le complexe le decapeptide Pro-rich (APTMPPPLPP).

SH3 domain:

Molecular velcro: mediate interactions between kinases & regulatory proteins present in great variety of proteins

- receptor Tyrosine Kinases
- non-Receptor Tyrosine Kinases,
- adaptor proteins (ex. Grb2)
- structural proteins (myosin, spectrin)

bind Pro-rich peptides



Relaying the signal:

Binding Modules, Adaptors, GEF, GAP

- SH2 domains mediate signal Transduction
- PTB domains bind pY-containing peptides
- SH3 domains bind Pro-rich peptides

Other Binding Modules

WW domain (2 Trp residues)

Plekstrin homology domain (PH domain)

PDZ domain

Relay:

Grb2, Shc & IRS:

- adaptor proteins
- recruit Sos to the vicinity of Ras

Ras is activated by **RTK** via **Grb2-SOS** complex

Activation of Ras

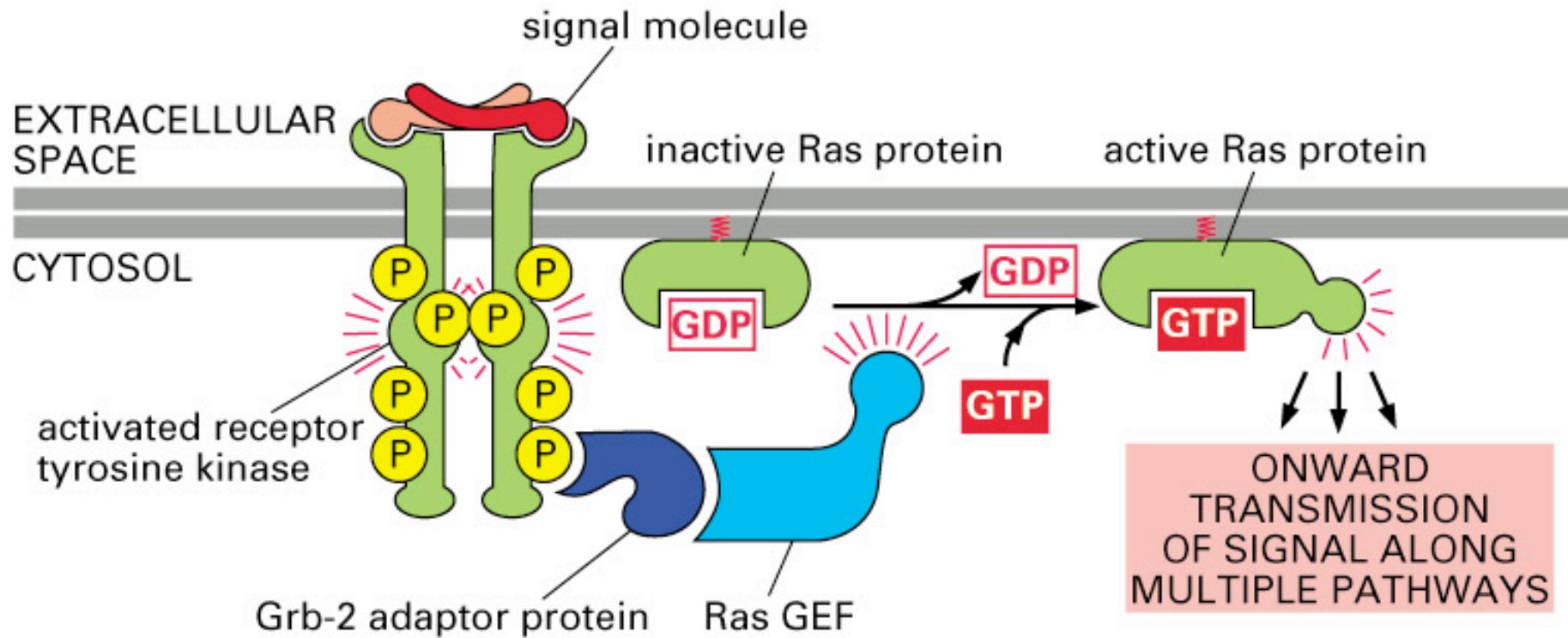
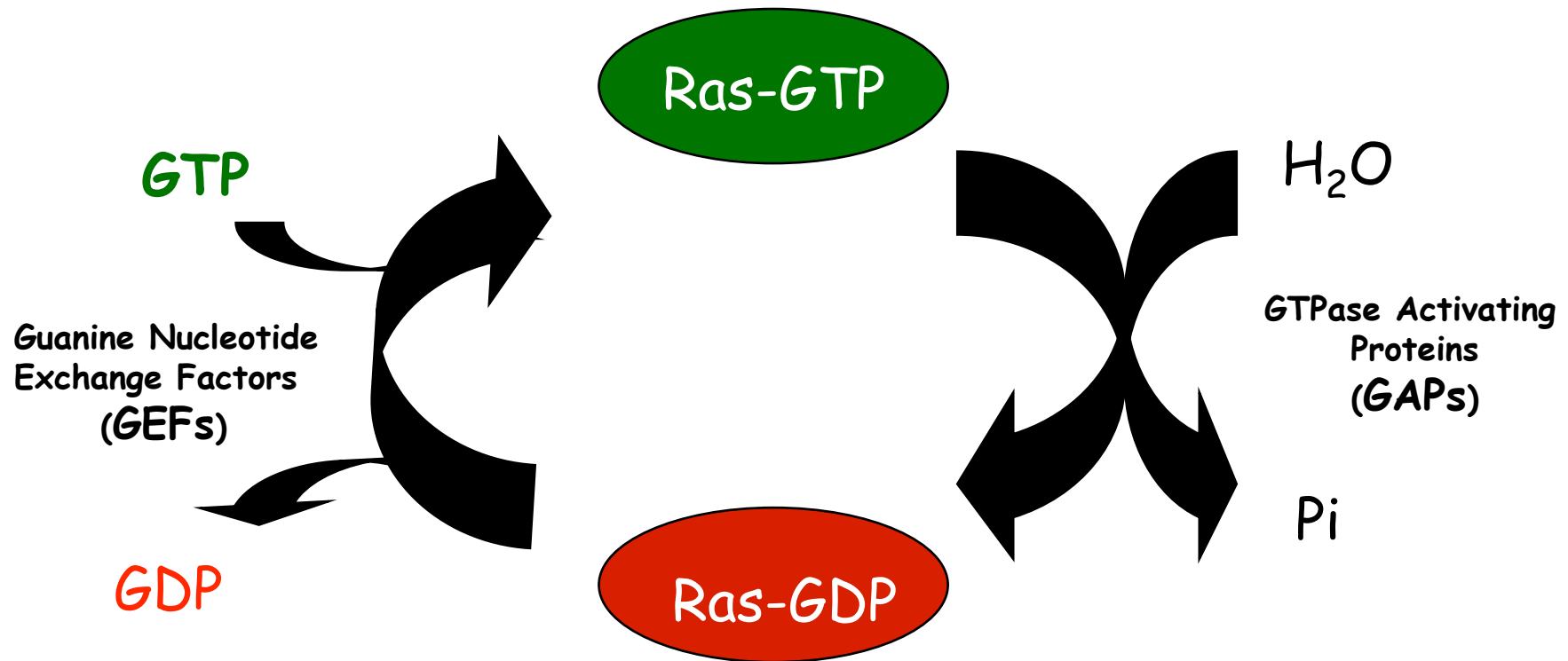


Figure 15–55. Molecular Biology of the Cell, 4th Edition.

Ras GTPase Cycle



-GAPs discovered biochemically

-GEFs discovered genetically- first in yeast and then drosophila

Ras Superfamily

<u>Ras</u>	<u>Rho</u>	<u>Rab</u>	<u>Arf</u>	<u>Ran</u>
H-Ras	RhoA	Rab _{1-N}	Arf ₁₋₆	Ran
N-Ras	RhoB			
K-Ras	RhoC			
TC21	RhoG			
	RhoE			
Rap1				
Rap2	CDC42			
R-Ras	Rac1			
	Rac2			
RalA				
RalB				

Functions of Ras Proteins

- 1) Promote Cell Proliferation
 - fibroblasts, epithelial cells, lymphocytes
 - mediate actions of growth factors
- 2) Promote Cell Differentiation
 - neuronal progenitor cells (PC12)
 - mediate action of neurotrophins
- 3) Contribute to Differentiated Cell Functions
 - CNS neurons
 - mediate effects of calcium signaling

Complexe Ras-GDP-GAP43-AIF3

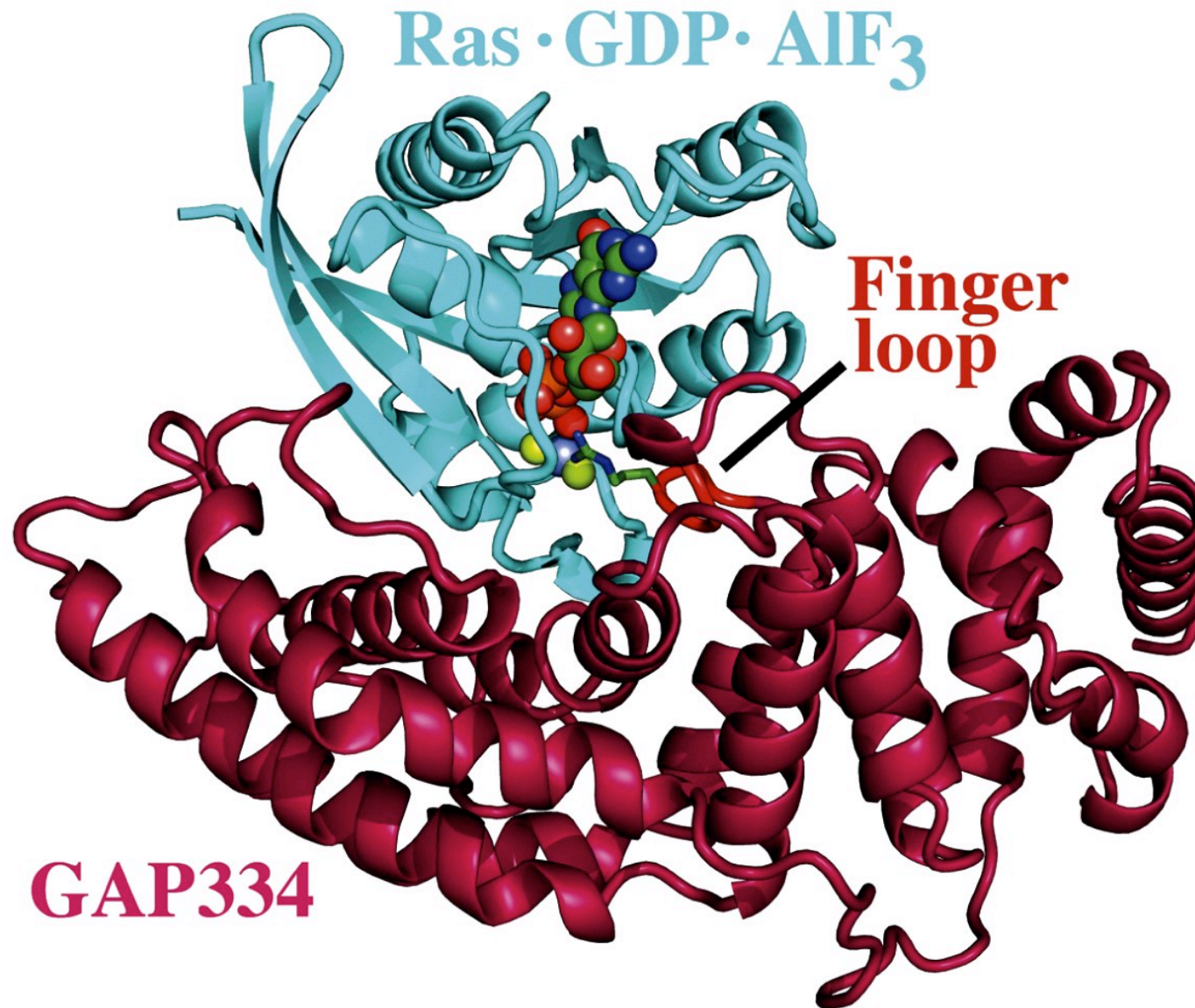
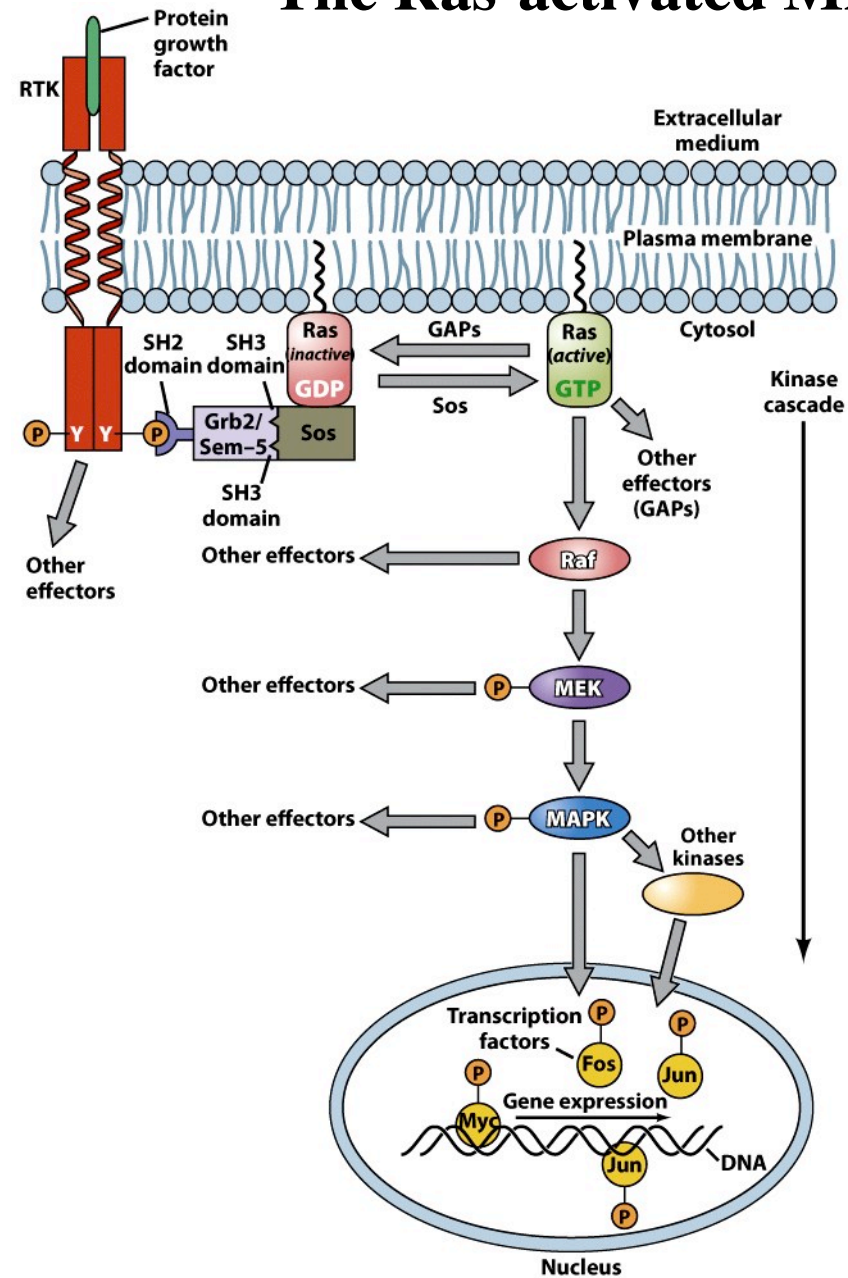


Figure 13-10

The Ras-activated MAP kinase cascade



Raf

Mek

Erk

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Figure 13-7

MAP KINASE

- The mitogen-activated protein kinase (MAPK) pathways are typically comprised of a three-member protein kinase cascade.
- Specificity of MAPK responses is achieved by activation of different three-kinase modules.
- There are at least three sets of mammalian MAPK modules.
 - the extracellular-signal-regulated kinases (ERKs),
 - the Jun N-terminal kinases (JNKs)
 - the p38 kinases.
- As a group, the MAPKs are major players in mediating a variety of signals for cell proliferation and differentiation.

Cascade MAP-kinase

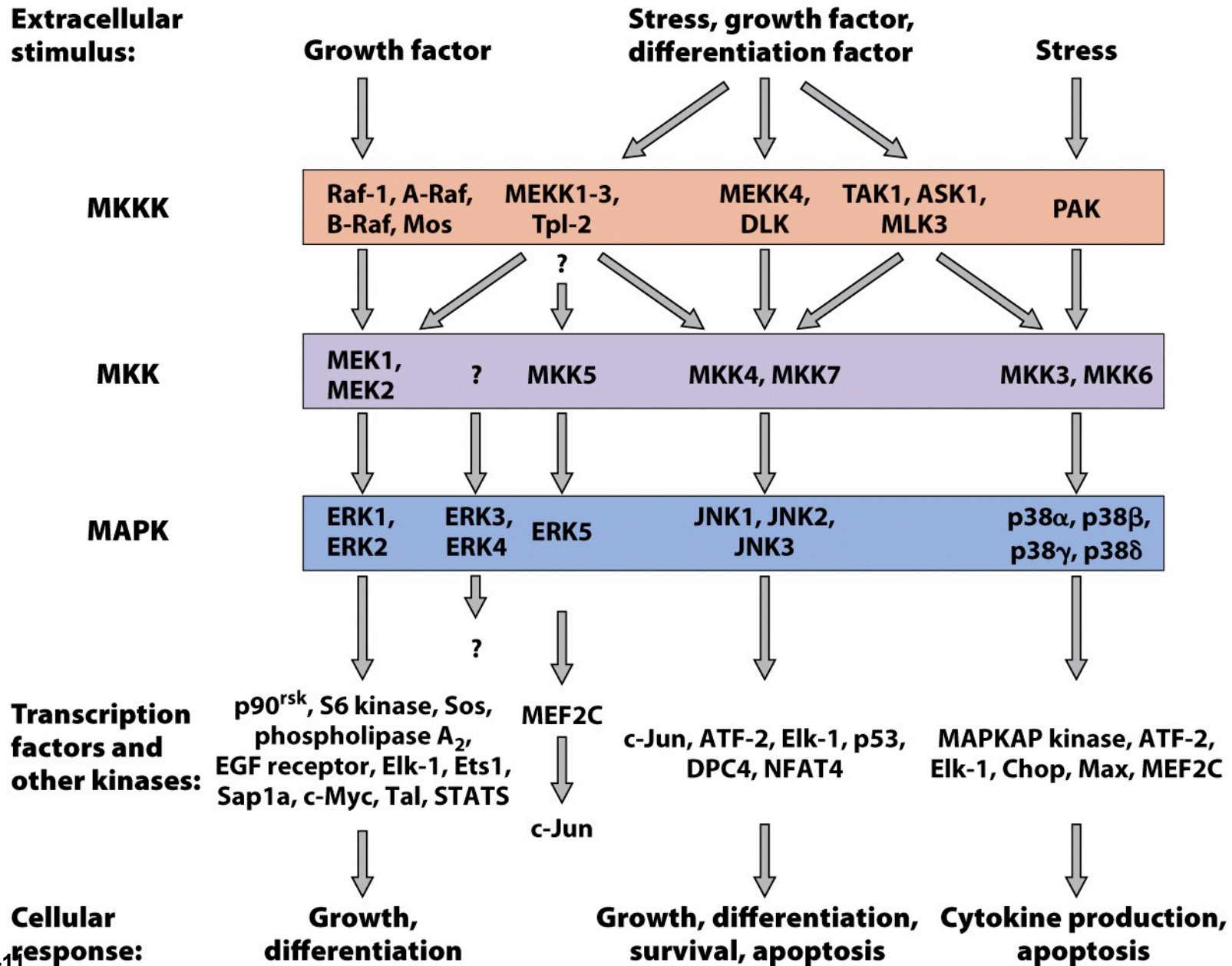


Figure 13-11

Scaffolding proteins help organize MAPKs

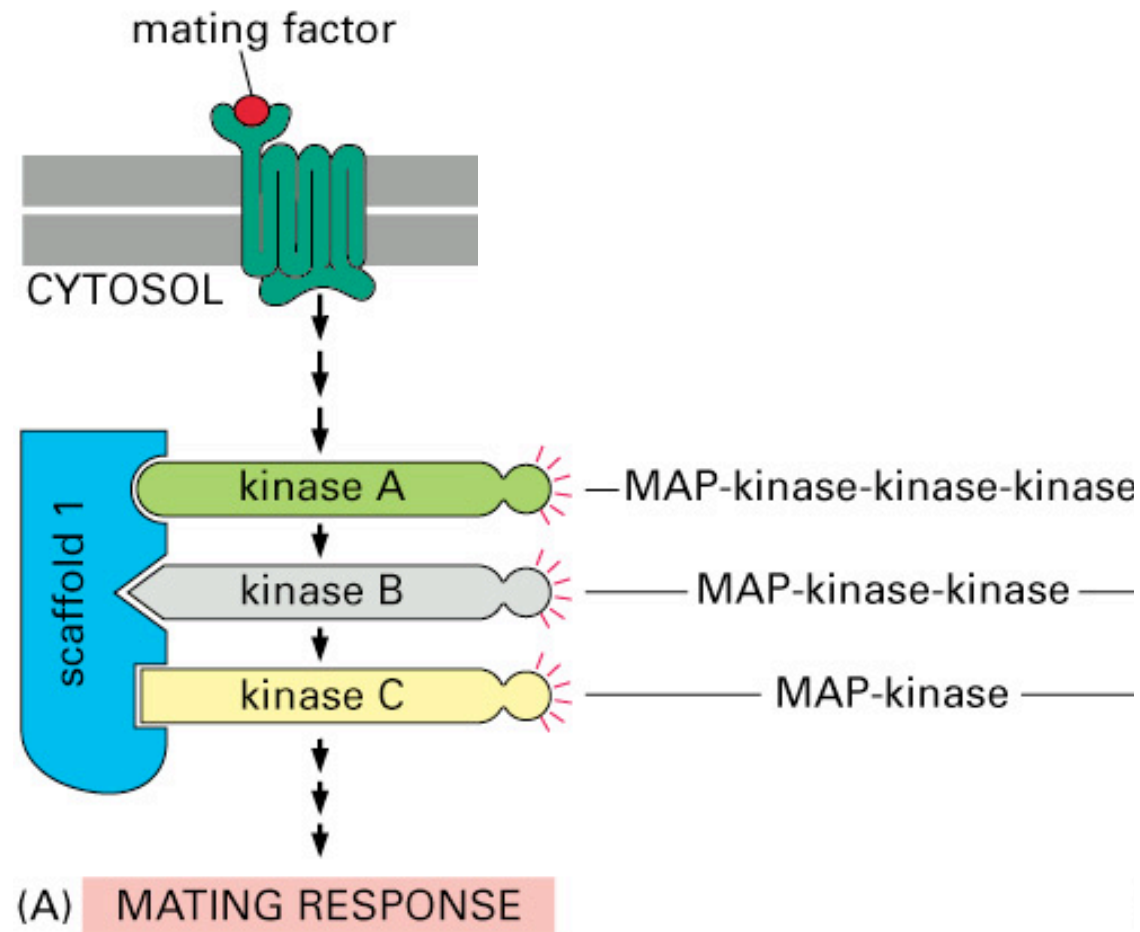
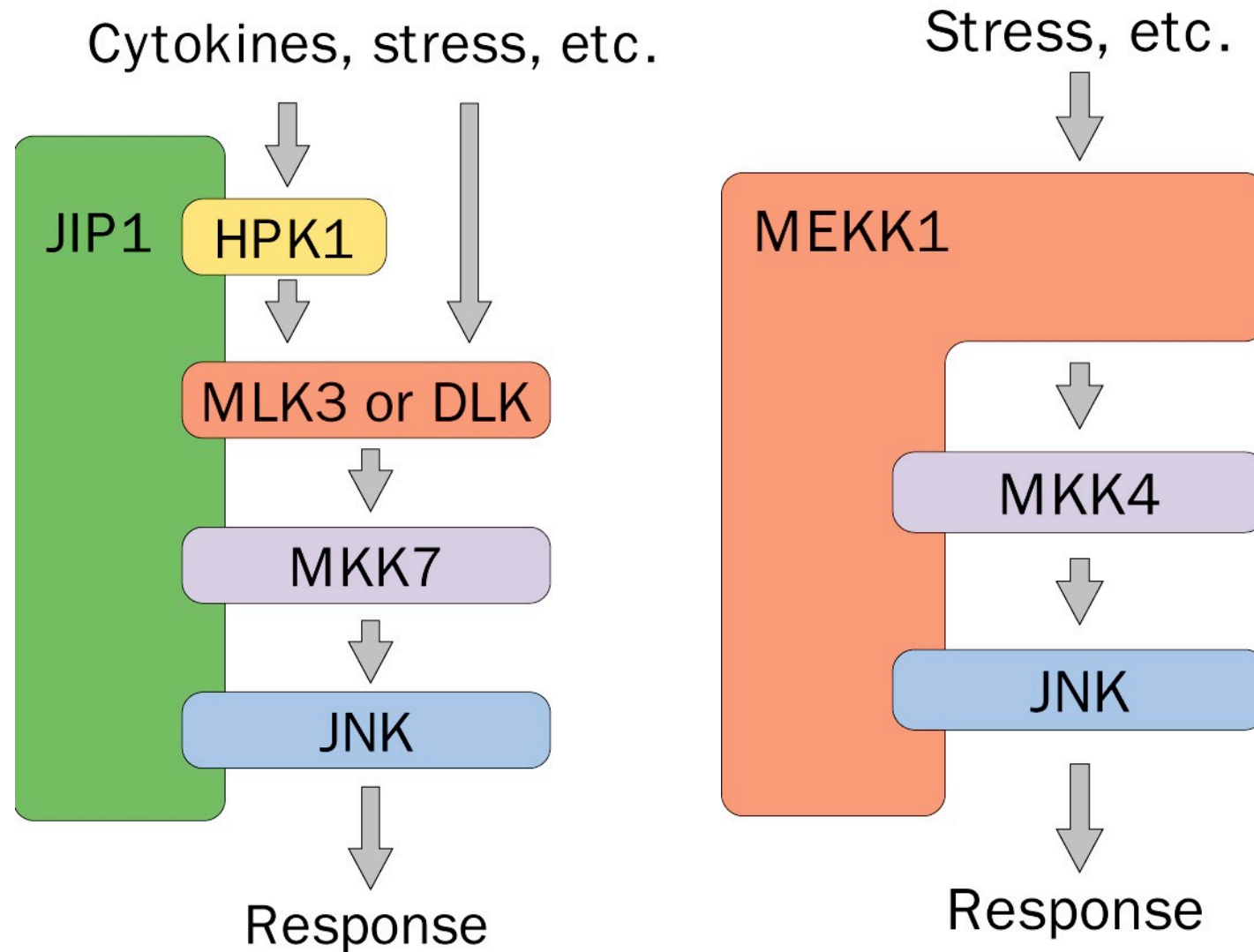


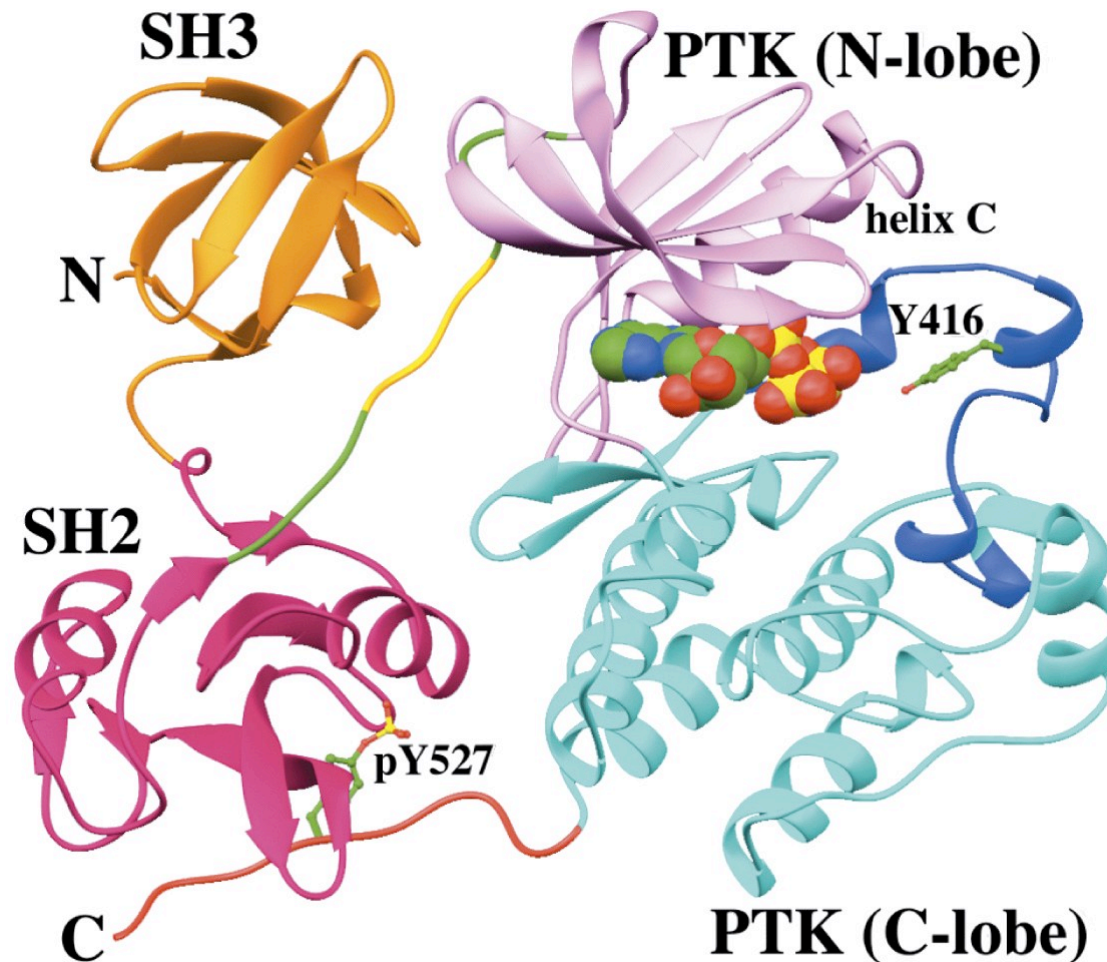
Figure 15-57. Molecular Biology of the Cell, 4th Edition.



Scaffold proteins that modulate mammalian MAP kinase cascades. (a) JIP-1 (JNK-interacting protein). (b) MEKK1.

Structure de Src (= non-Receptor Tyrosine kinase)

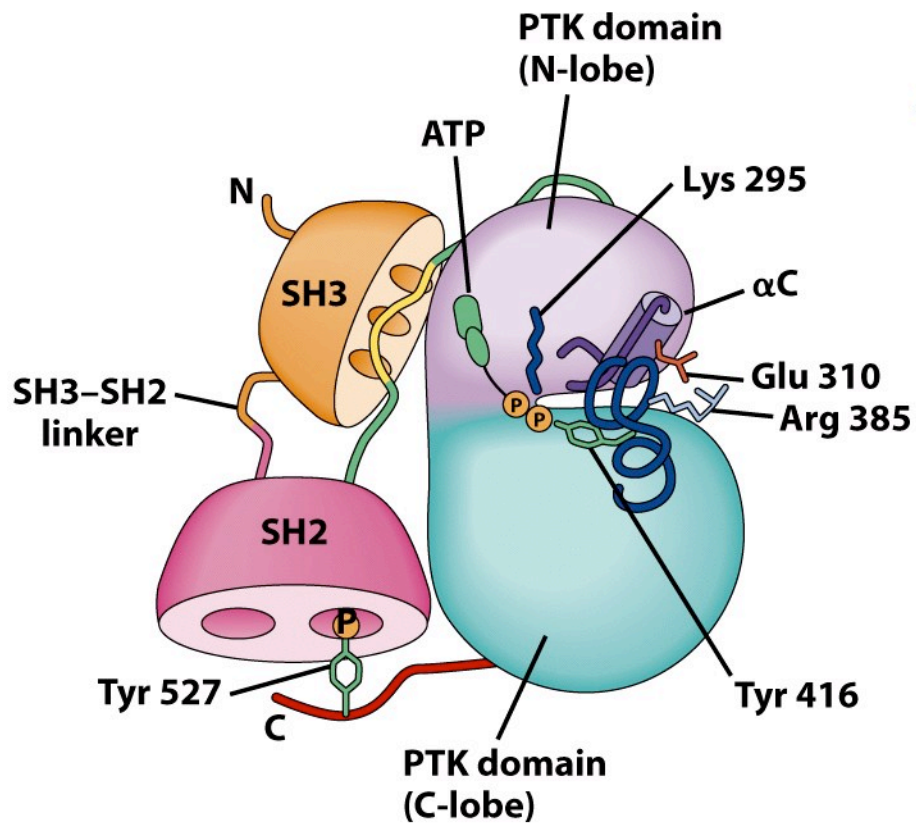
- Many NRTK are activated by tyrosine kinase-associated receptors of the Src family
- Examples of TK-associated receptors: **Src**, Fyn, Lck
- Autoinhibitory mechanisms of Src



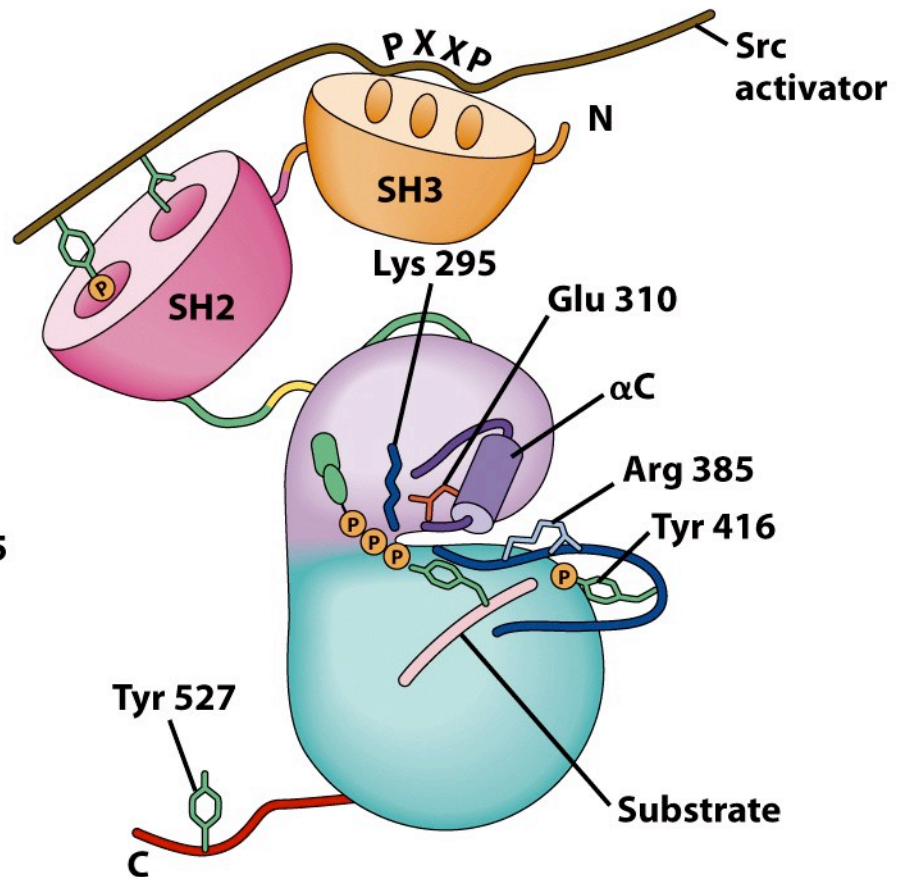
Structure de Src (=non Receptor Tyrosine kinase)

Modèle d'activation

Autoinhibited Form



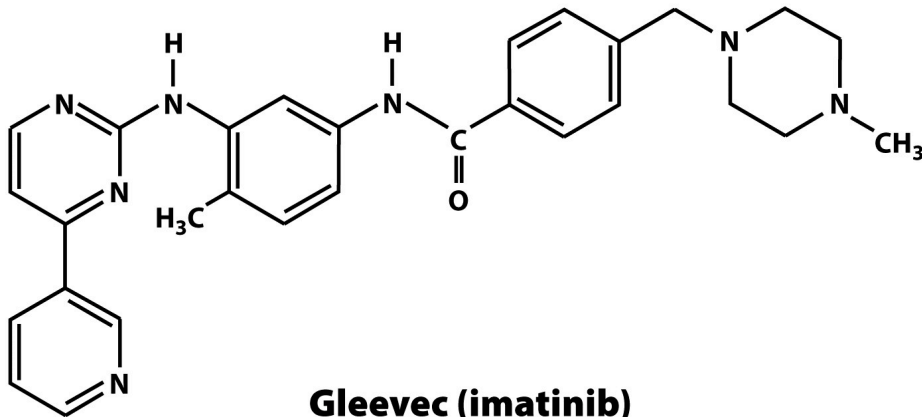
Active Form



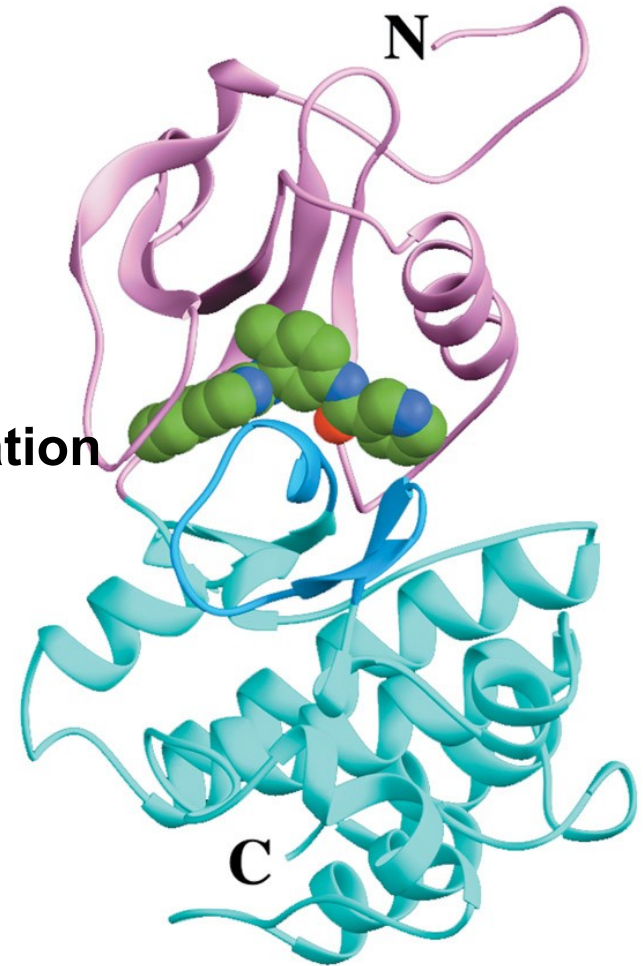
Abl

Abl = Protein Tyrosine Kinase - role in cancer

inhibitor of abl (*gleevec*) may inhibit cell proliferation

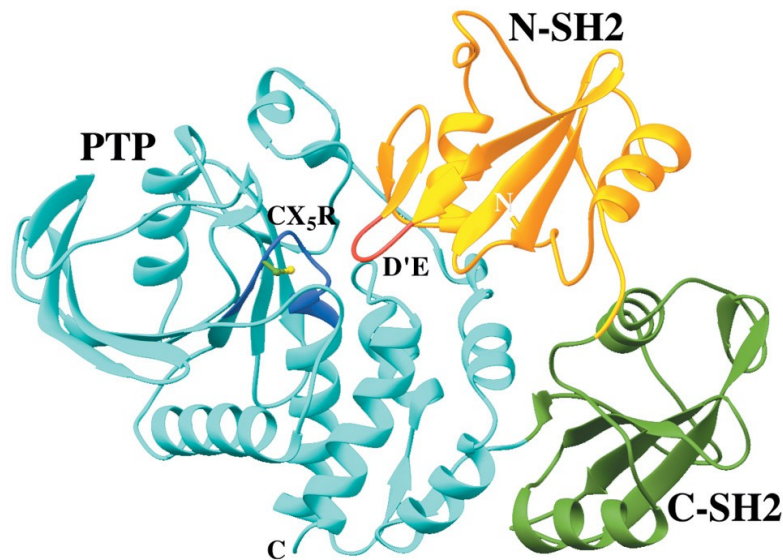


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Structure of the Abl PTK domain in complex with a truncated derivative of gleevec (anticancer drug).

PROTEIN TYROSINE PHOSPHATASES



Protein Tyr Phosphatases

- SHP-2

Protein Ser/Thr Phosphatases

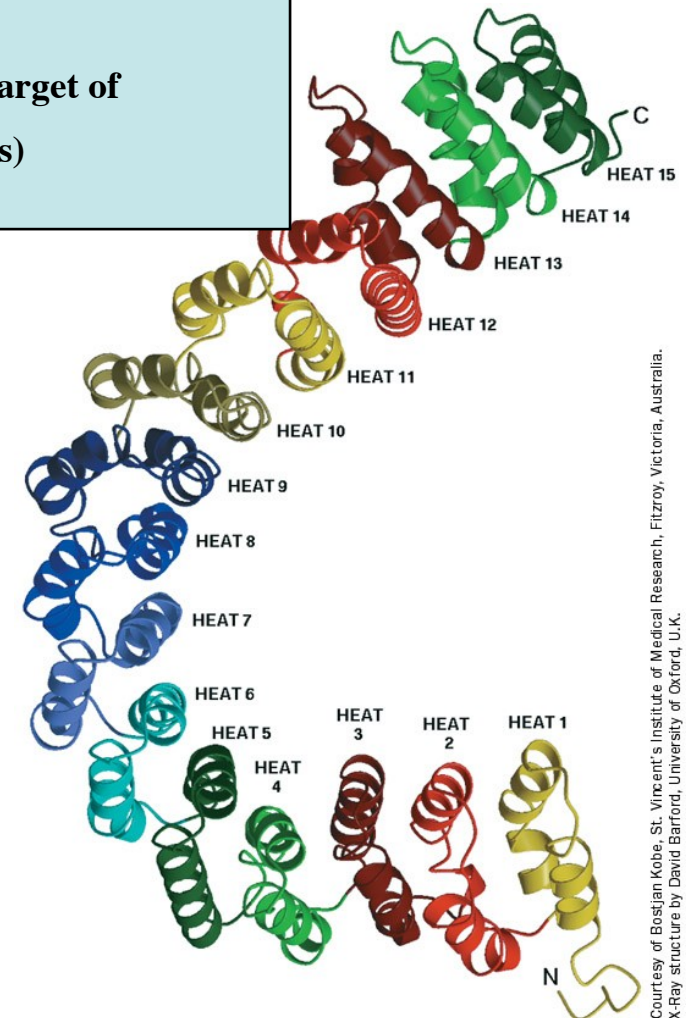
- PP1
- PP2A
- PP2B (=calcineurin / target of immunosuppressant drugs)
- PP2C

Protein tyrosine phosphatase SHP-2.

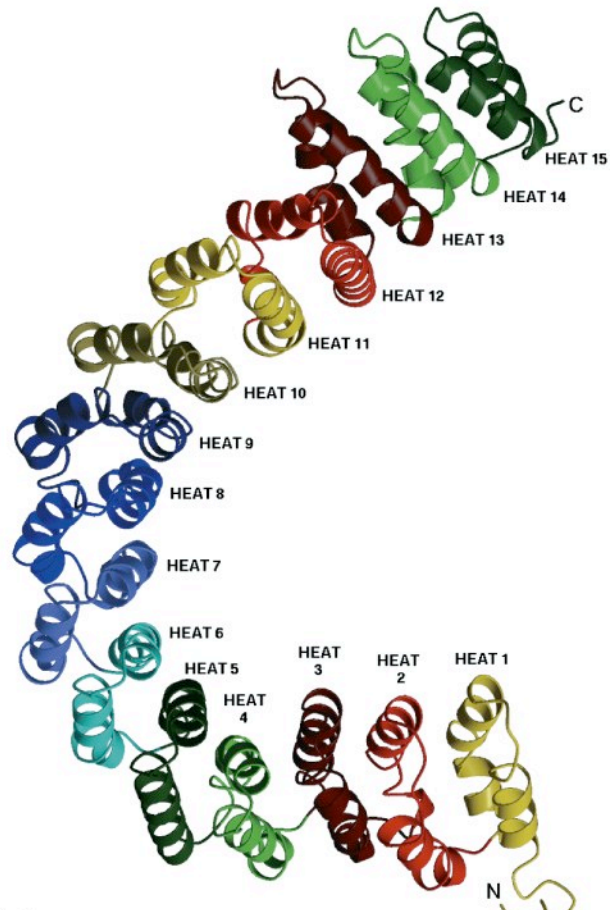
PP2A

- Structurally variable
- Functionally diverse
- Catalytic subunit
- Scaffold subunit (A)(PR65)
- Four different regulatory subunits (B, B', B'', B'''), bind to A & C subunits

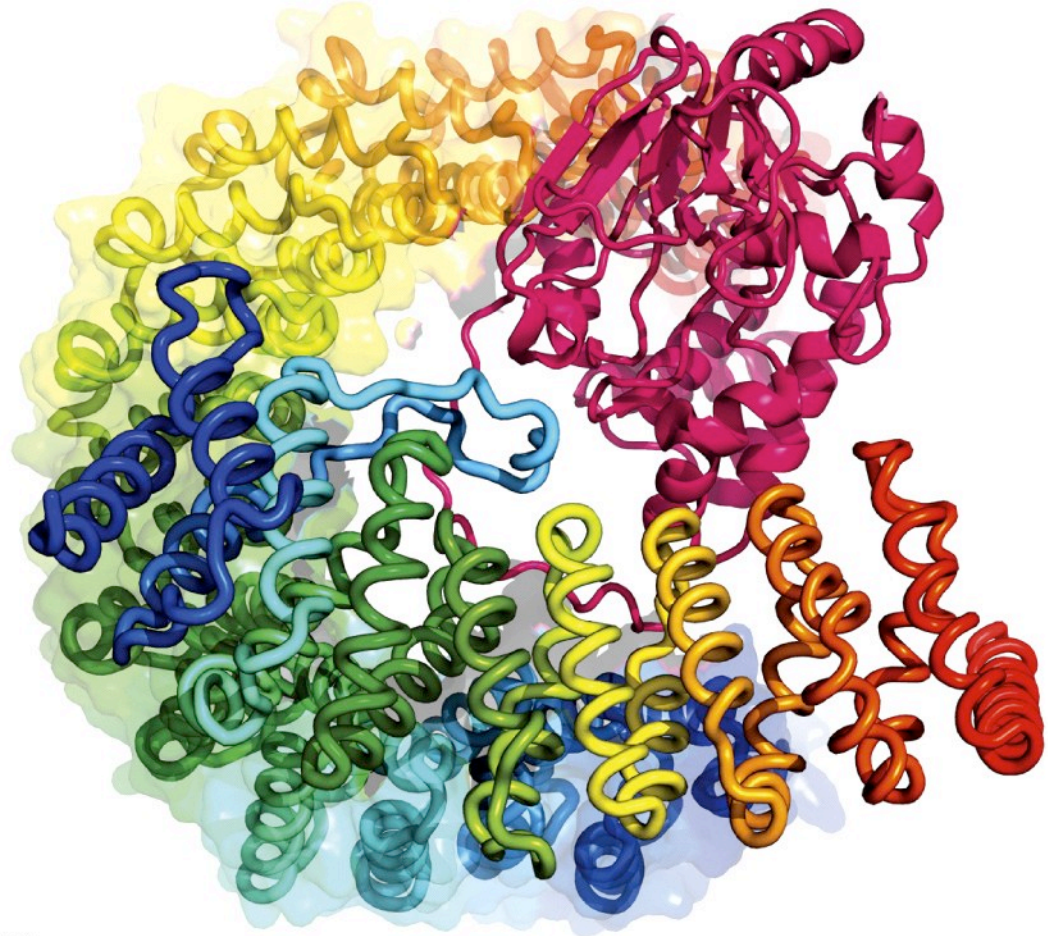
A subunit of PP2A.



PP2A

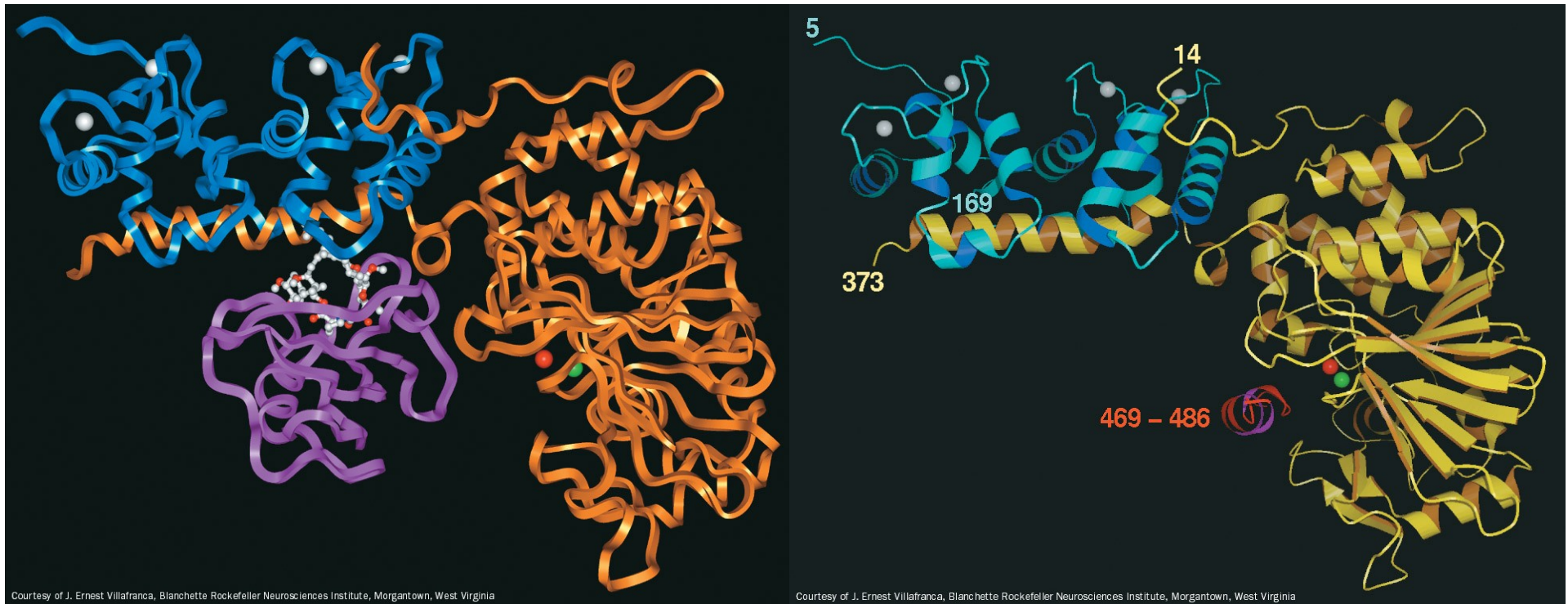


(a) Scaffold subunit: HEAT repeats



(b)

Courtesy of Bostjan Kobe, St. Vincent's Institute of Medical Research, Fitzroy, Victoria, Australia. X-Ray structure by David Barford, University of Oxford, U.K.



Calcineurin (=PP2B protein phosphatase)

(a) human FKBP12·FK506–CaN. (b) Human CaN with CaNA yellow, its autoinhibitory segment red, and CaNB cyan.

Cyclosporin & FK506: highly effective immunosuppressants

CALCINEURIN: target of immunosuppressive drugs

ex. immunophilins (cyclophilins, FKBP12, etc)

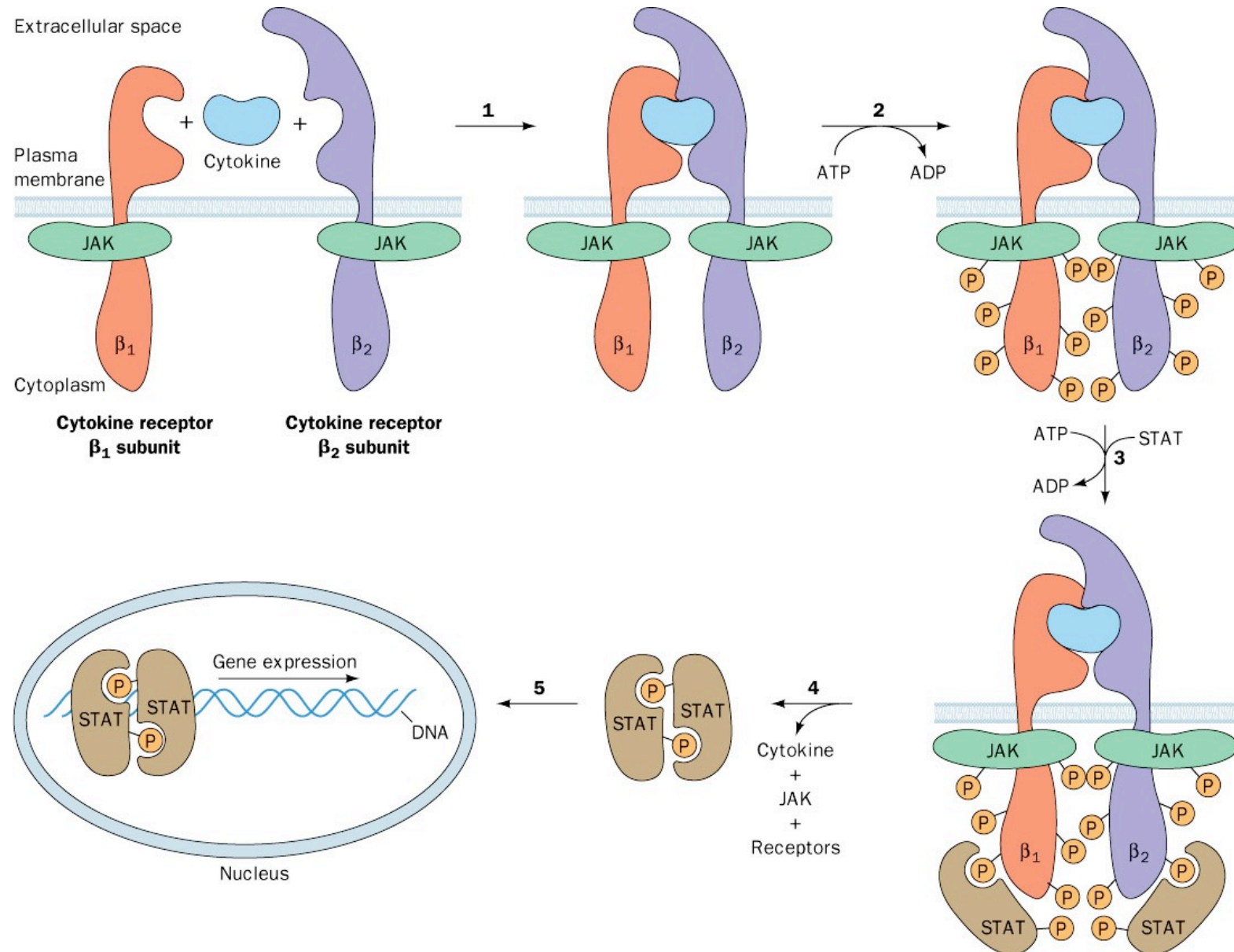
- subunits : Catalytic A (CaNA) + regulatory B (CaNB)
- *binds NFAT --> translocated into the nucleus --> gene expression*

JAK-STAT Pathway

JAK: = Janus kinases - contiennent 2 domaines Tyrosine Kinase

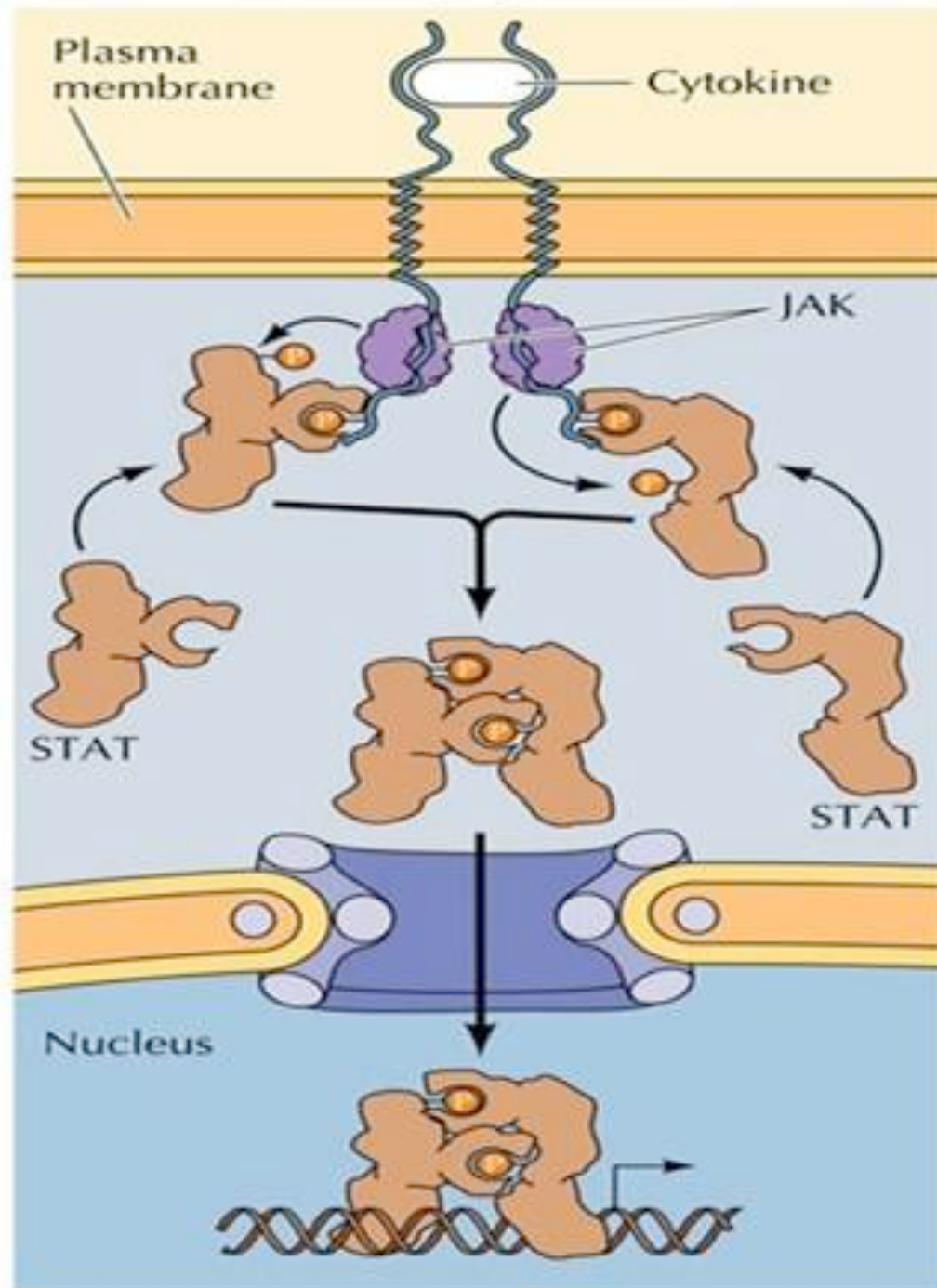
STAT: Signal Transducers and Activators of Transduction
= Transcription Factors, activated by JAK

1. - *Ligand binding dimerizes the receptor ($\beta 1$ and $\beta 2$ subunits)*
2. - *Receptor dimers bind JAK and induce phosphorylation of JAK*
3. - *Phosphorylated JAK phosphorylate the Receptor Subunits*
4. - *Phosphorylated Receptor can phosphorylate STAT*
5. - *Phosphorylated STAT dimerizes*
6. - *Dimeric, phosphorylated STAT moves to the nucleus and act as Transcription Factors*
7. - *---> gene expression*

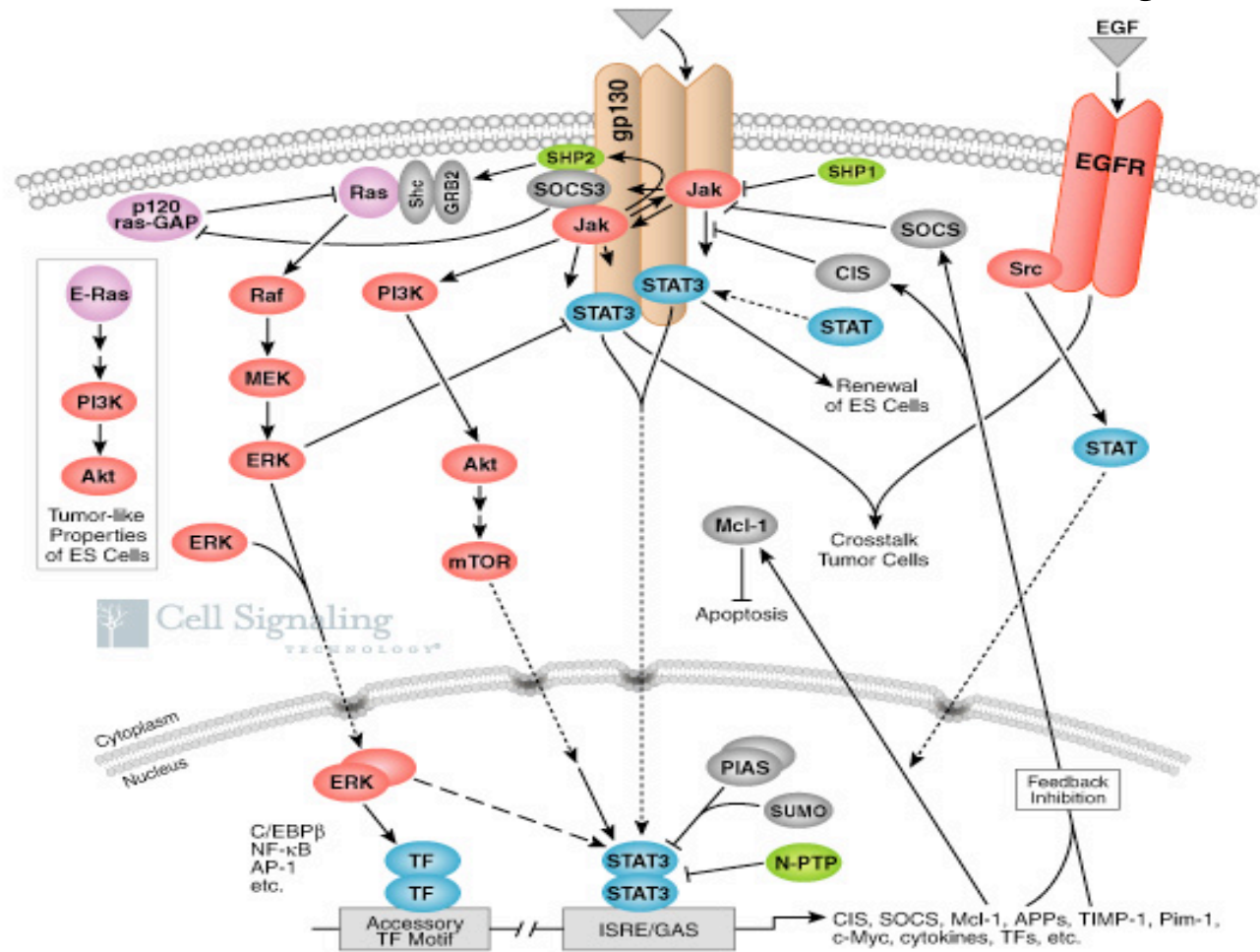


The JAK-STAT pathway for the intracellular relaying of cytokine signals.

JAK-STAT

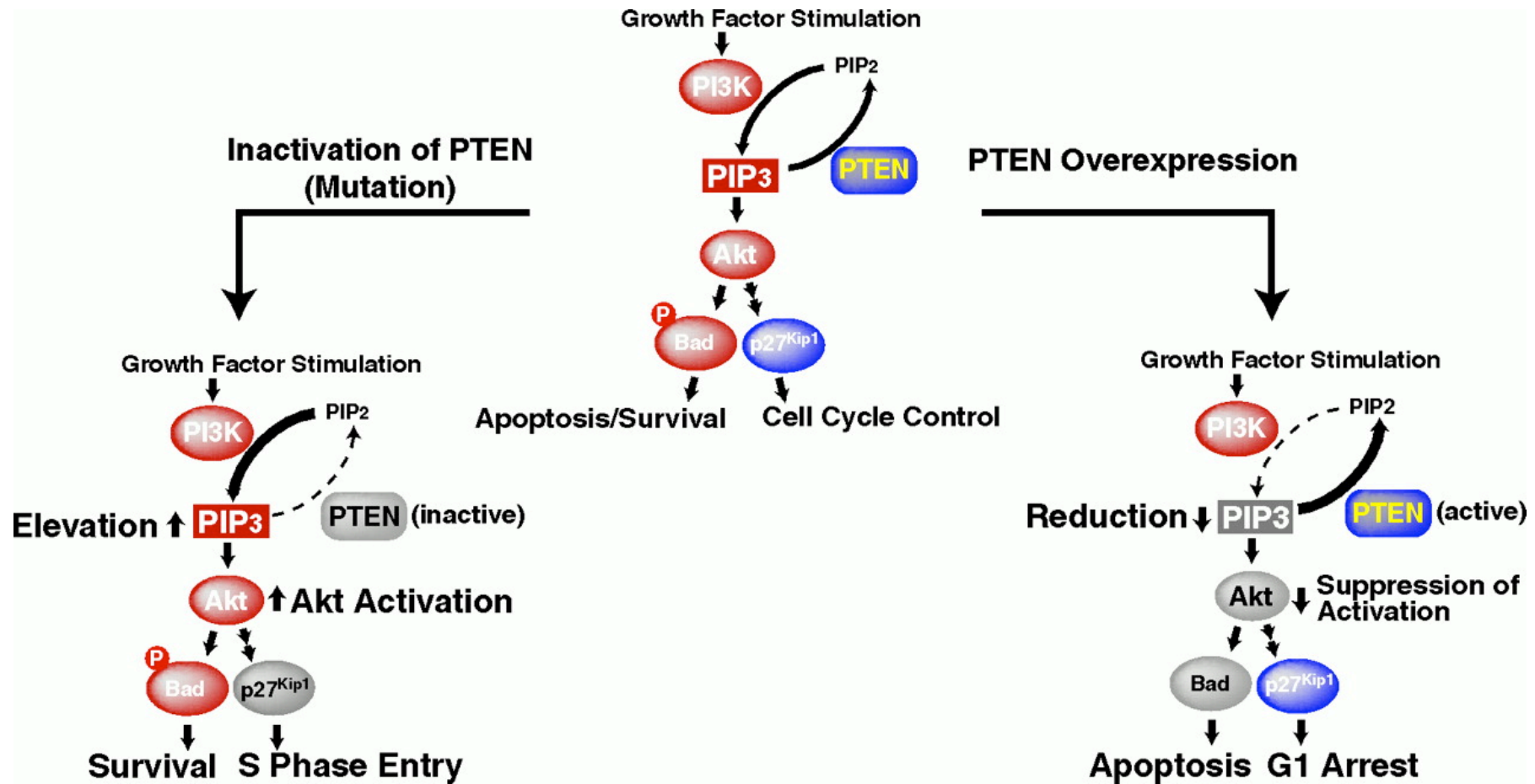


JAK-STAT Pathway

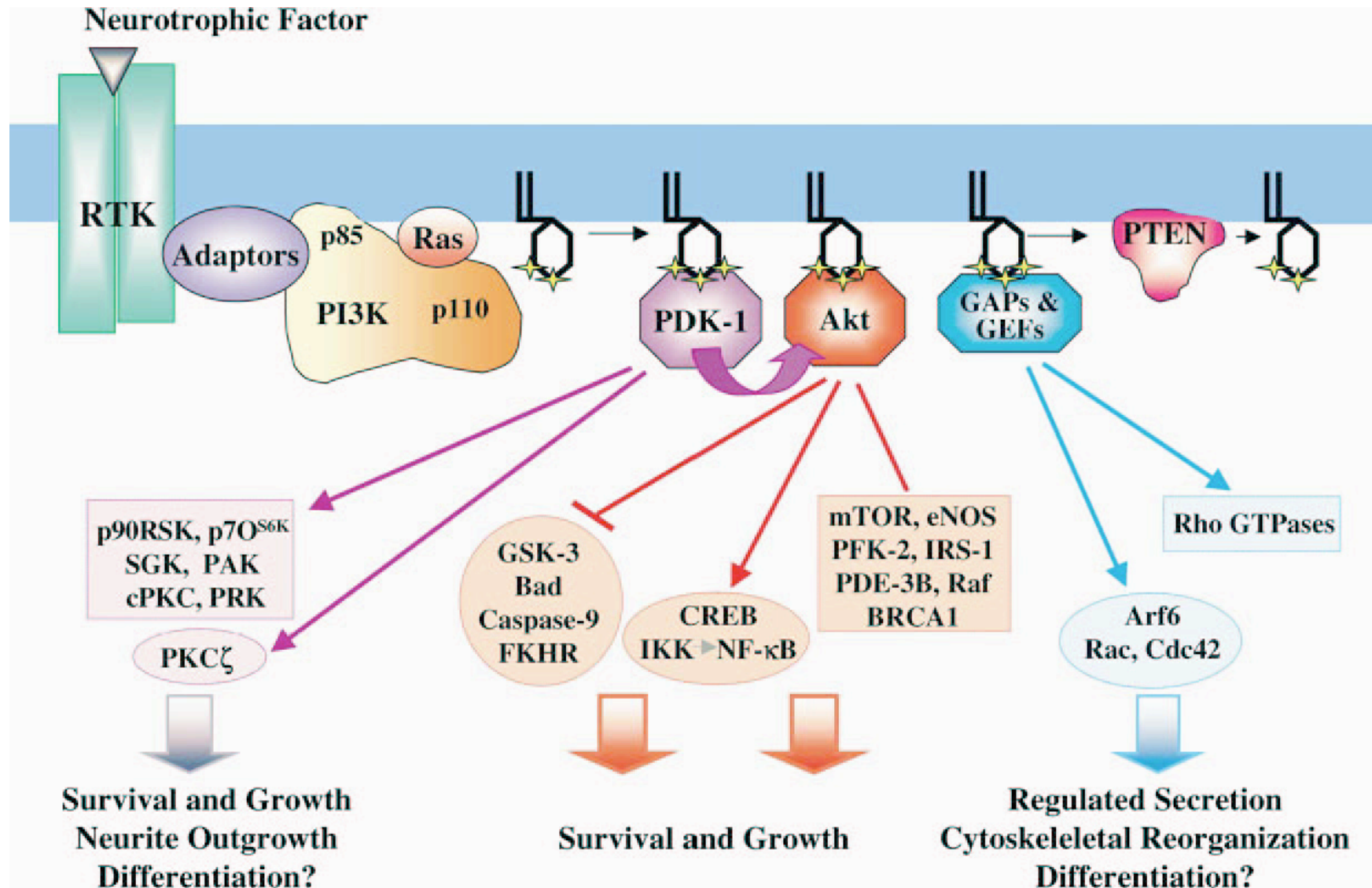


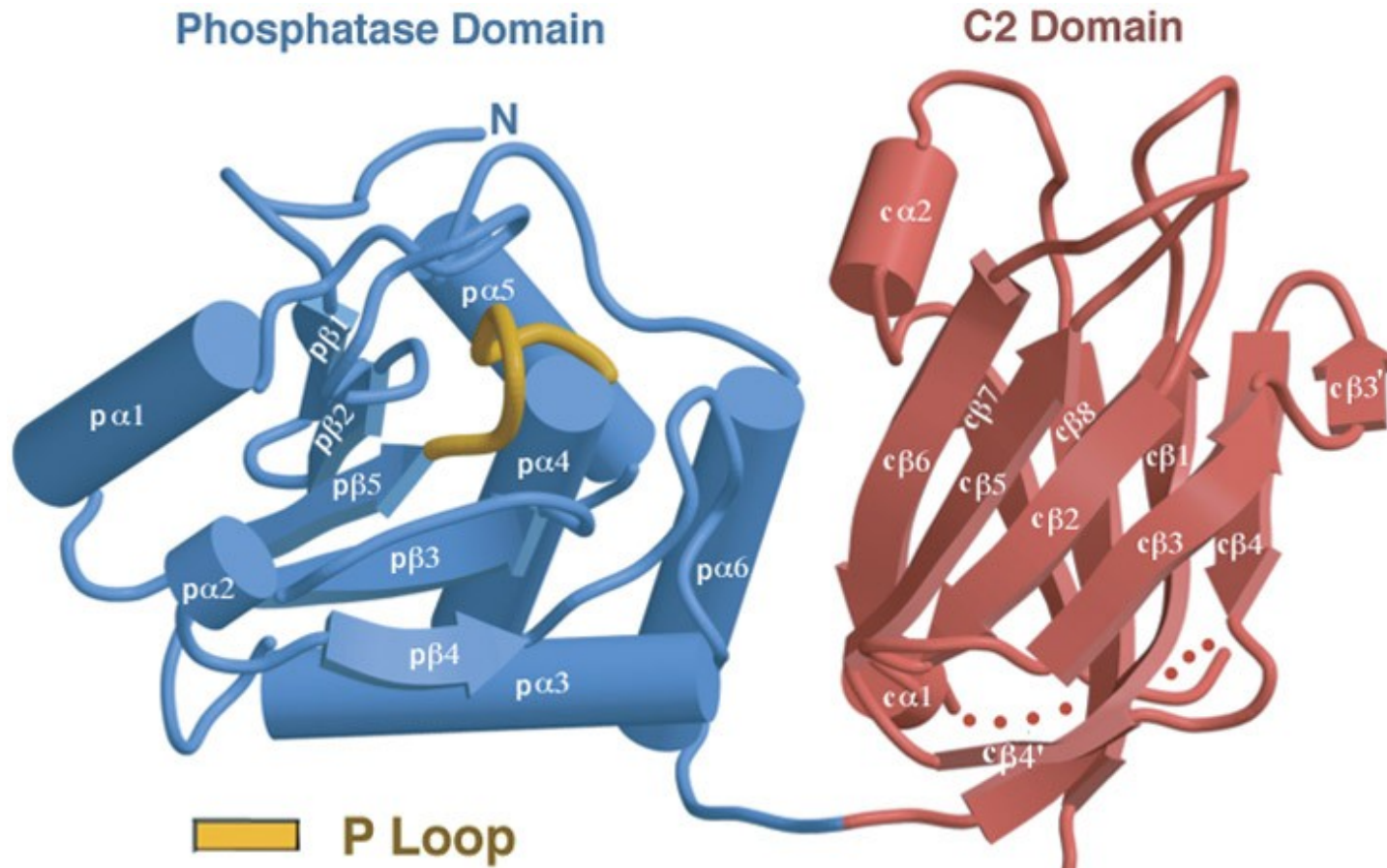
KEY





PI3K





Structure of PTEN (Phosphatase and Tensin homolog).

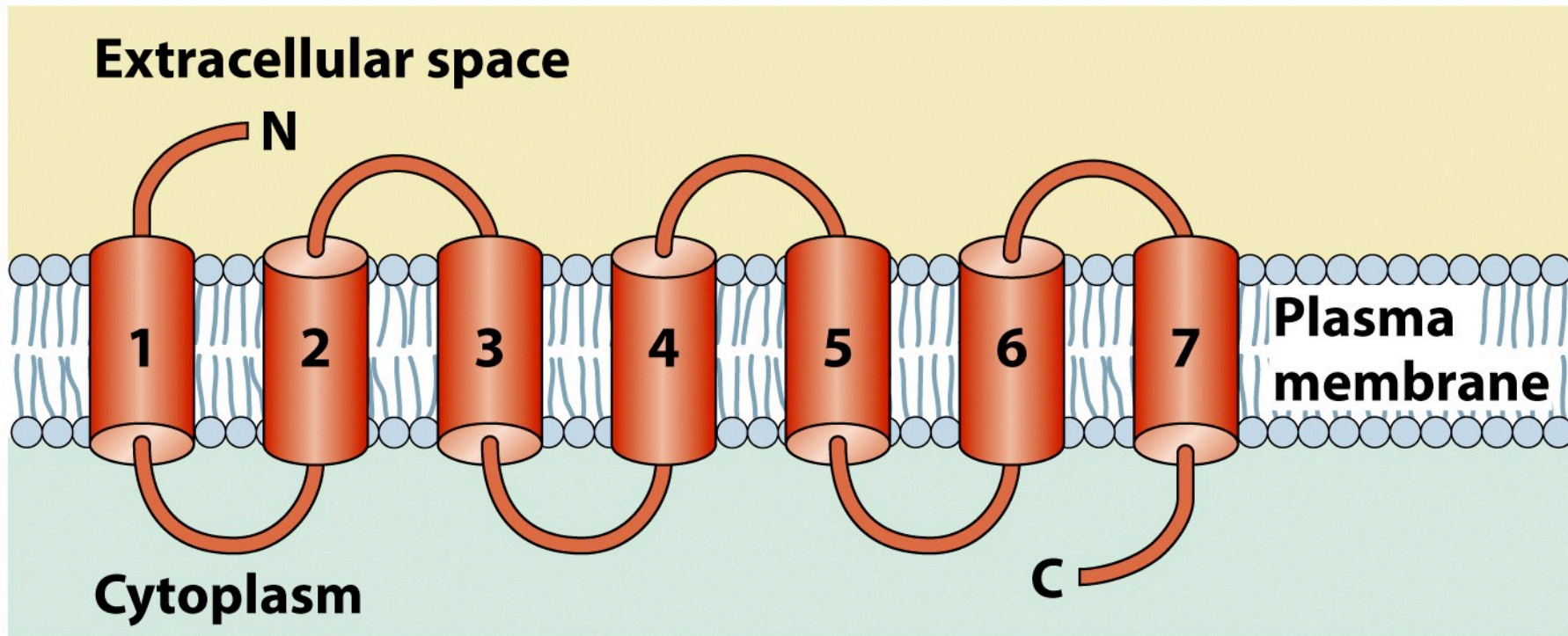
Tensin = cytoskeletal actin binding protein

PTEN = inositol-polyphosphate phosphatase

- **Tumor suppressor**: loss of function results in cancer
- downregulates Akt - controls the levels of Ptd-Inositol-3,4-Phosphate
- dephosphorylates P-Ser & P-Thr

Heterotrimeric G-Proteins

GPCR = G-Protein Coupled Receptor



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Très grande famille de récepteurs
Recepteurs monomériques
7 domaines transmembranaires (7TM)

Figure 13-17

G-protein linked receptors

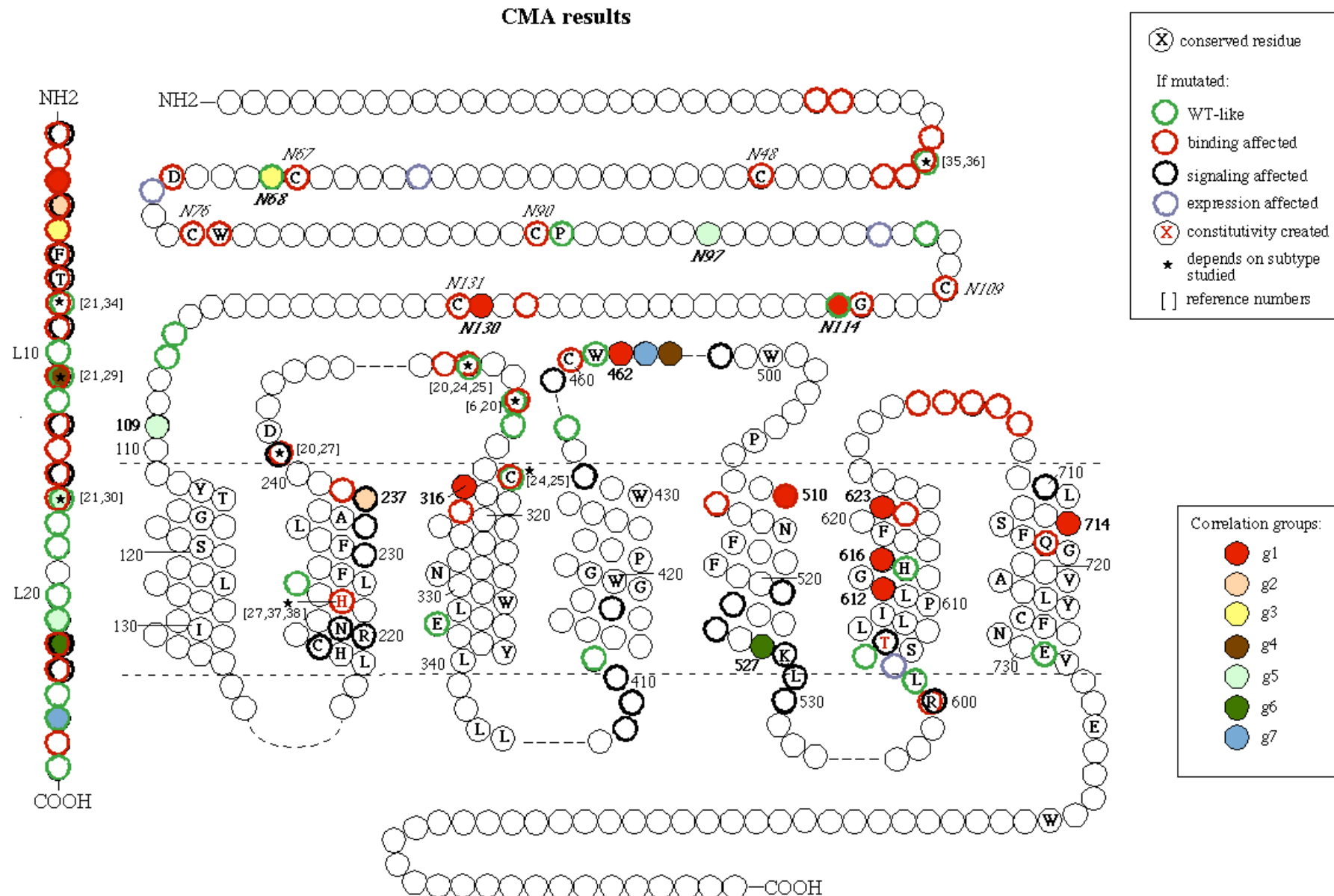
Ligand: Diverse ligands, such as epinephrine

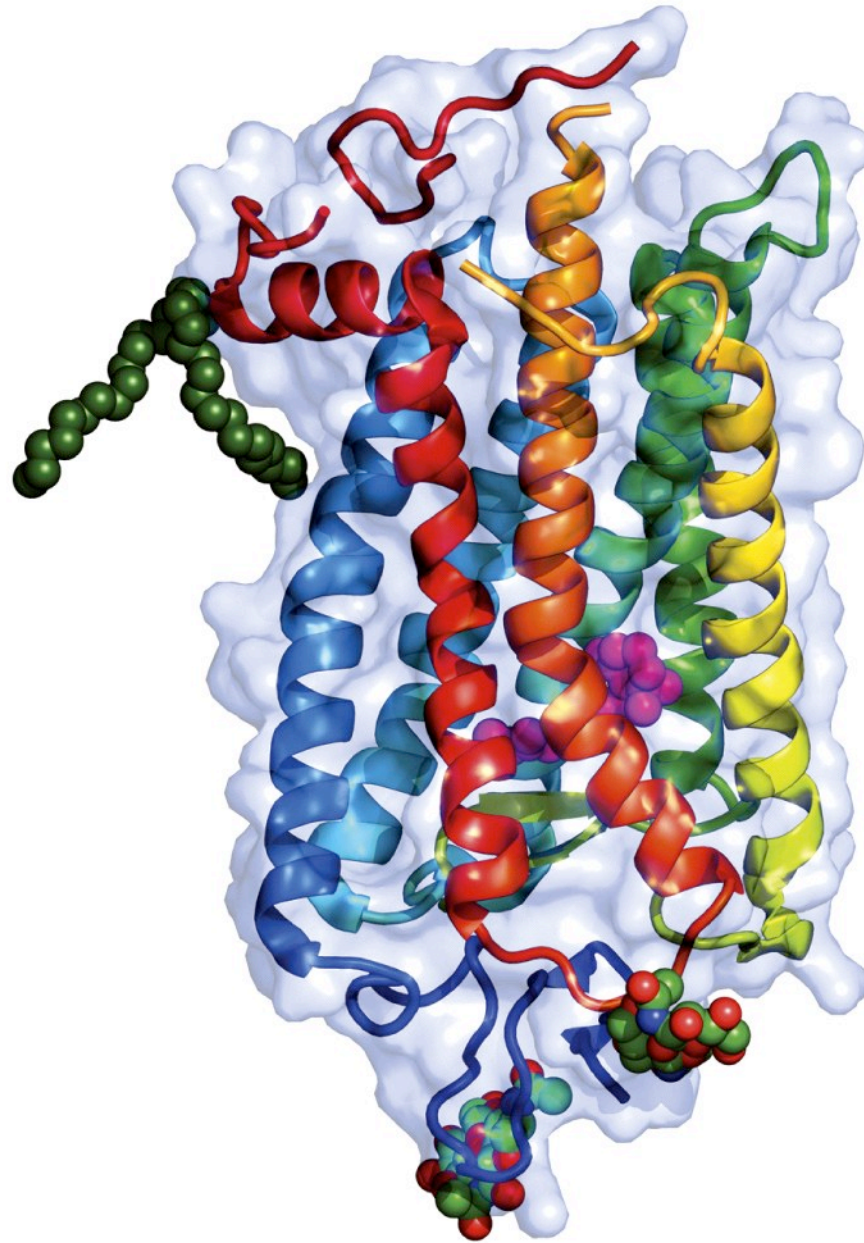
Receptor: Integral membrane protein with 7TM (7 transmembrane domains)

G-protein: trimeric protein (α , β , γ) attached to the cell membrane by lipid anchors

Effectors: Target proteins that show altered activity when they interact with activated G-protein subunits (α , or $\beta\gamma$)

G-protein coupled Receptor

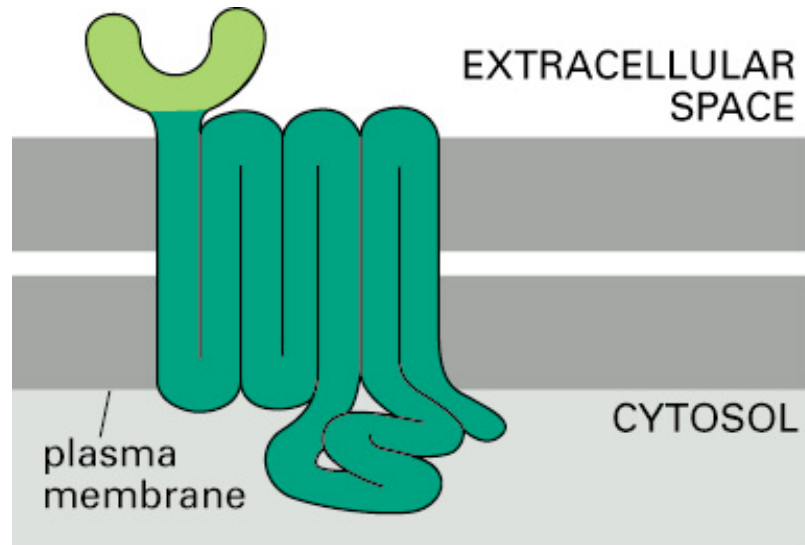




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Figure 13-18: Rhodopsine

G-protein linked receptors and G-proteins



Receptor

G-protein

Figure 15-26. Molecular Biology of the Cell, 4th

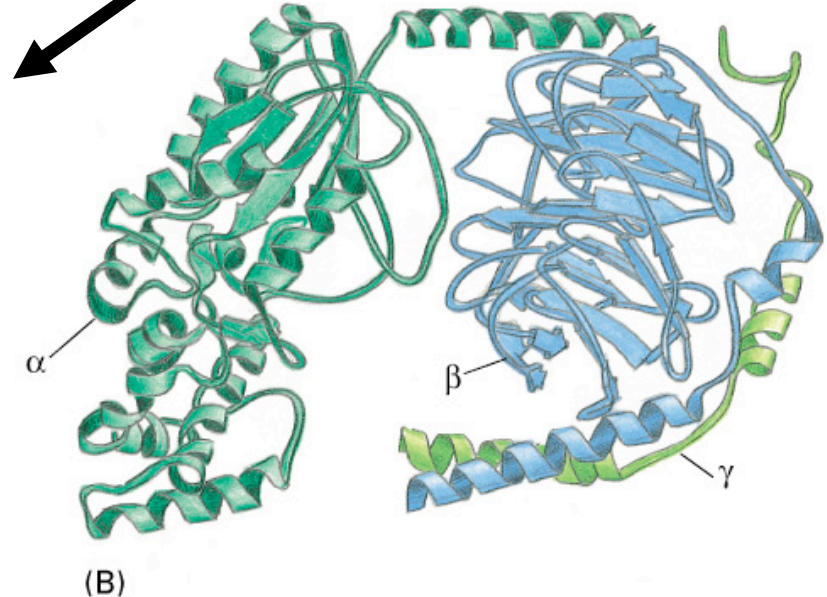
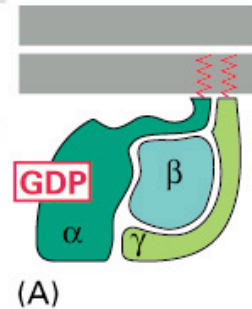
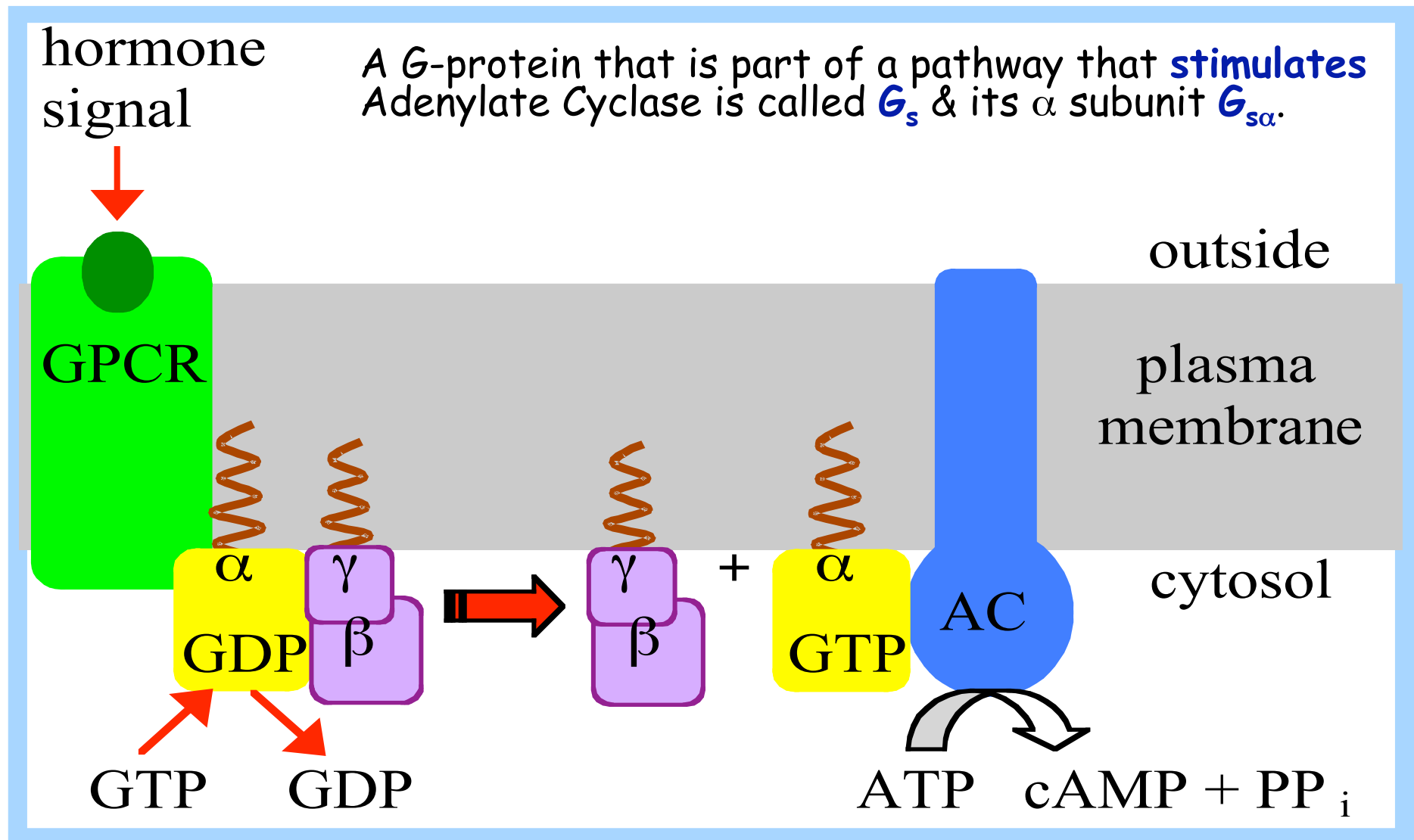
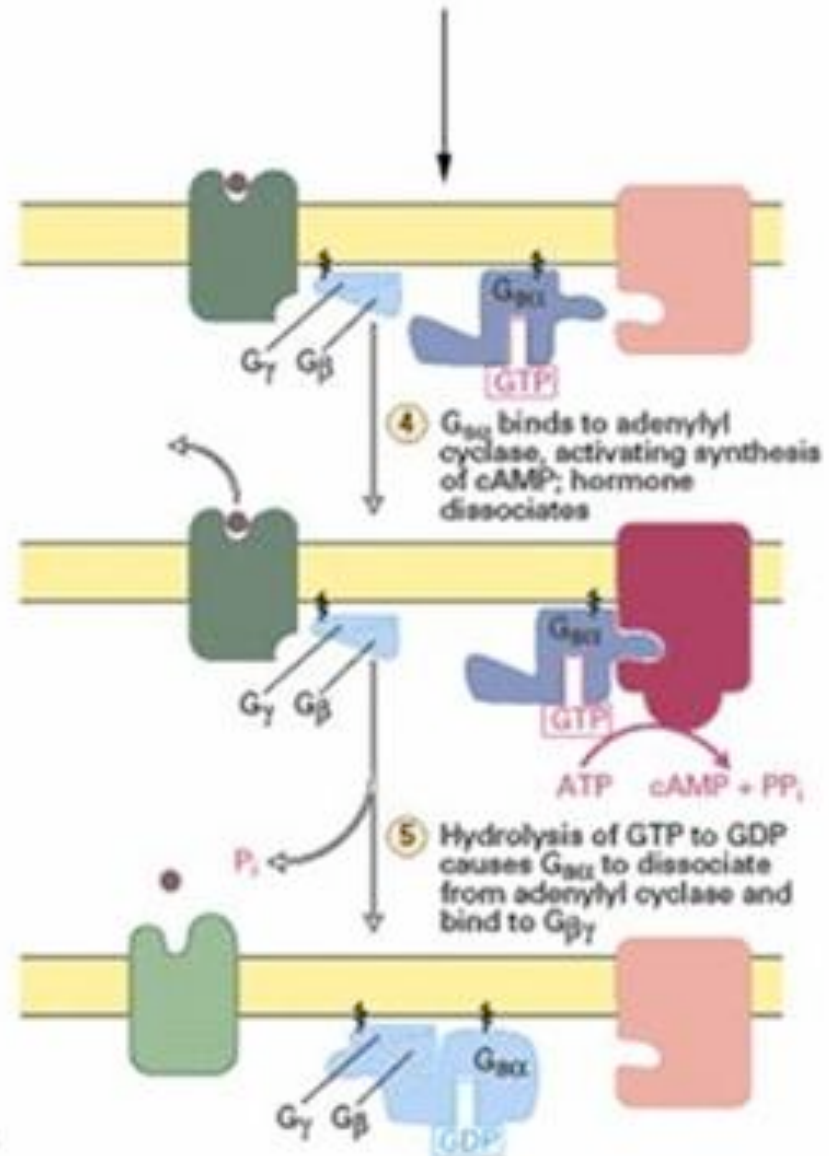
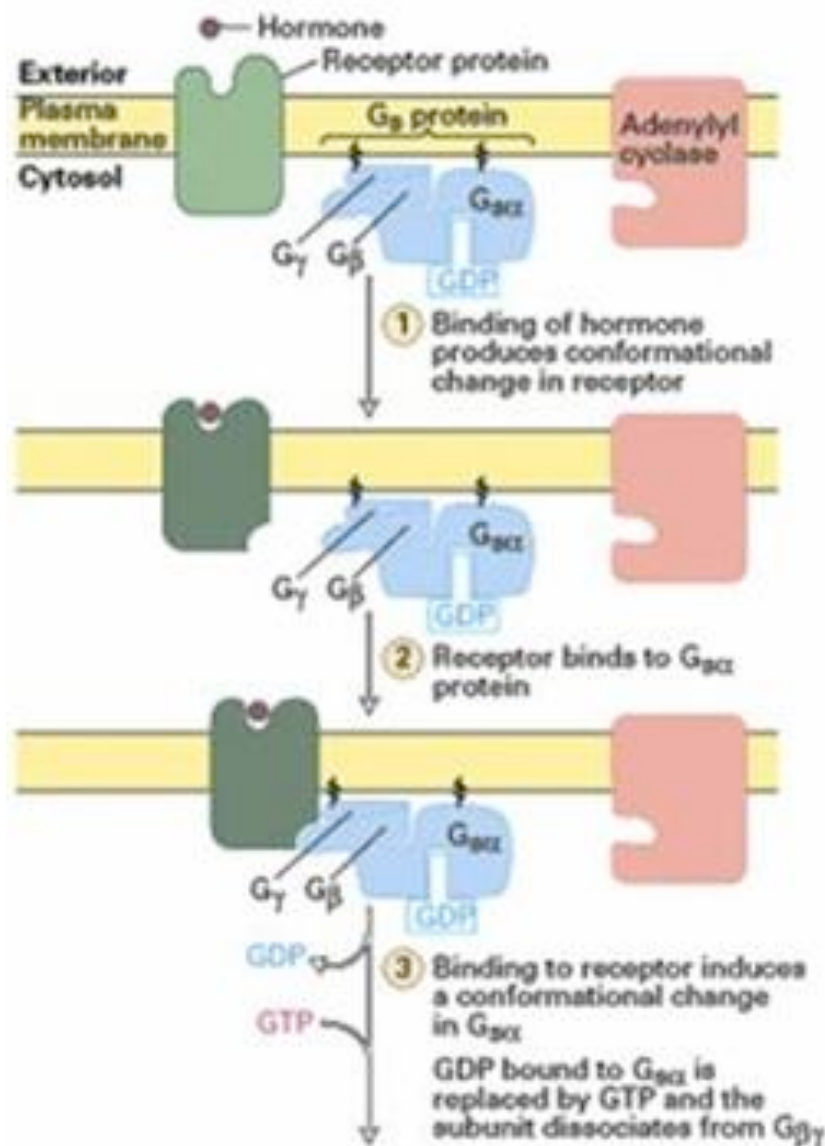


Figure 15-27. Molecular Biology of the Cell, 4th Edition.



The α subunit of a G-protein (G_α) binds GTP, & can hydrolyze it to GDP + P_i. α & γ subunits have covalently attached **lipid anchors** that bind a G-protein to the plasma membrane cytosolic surface. Adenylate Cyclase (AC) is a transmembrane protein, with cytosolic domains forming the catalytic site.

Gs & Gi Pathways



G-protein dissociation

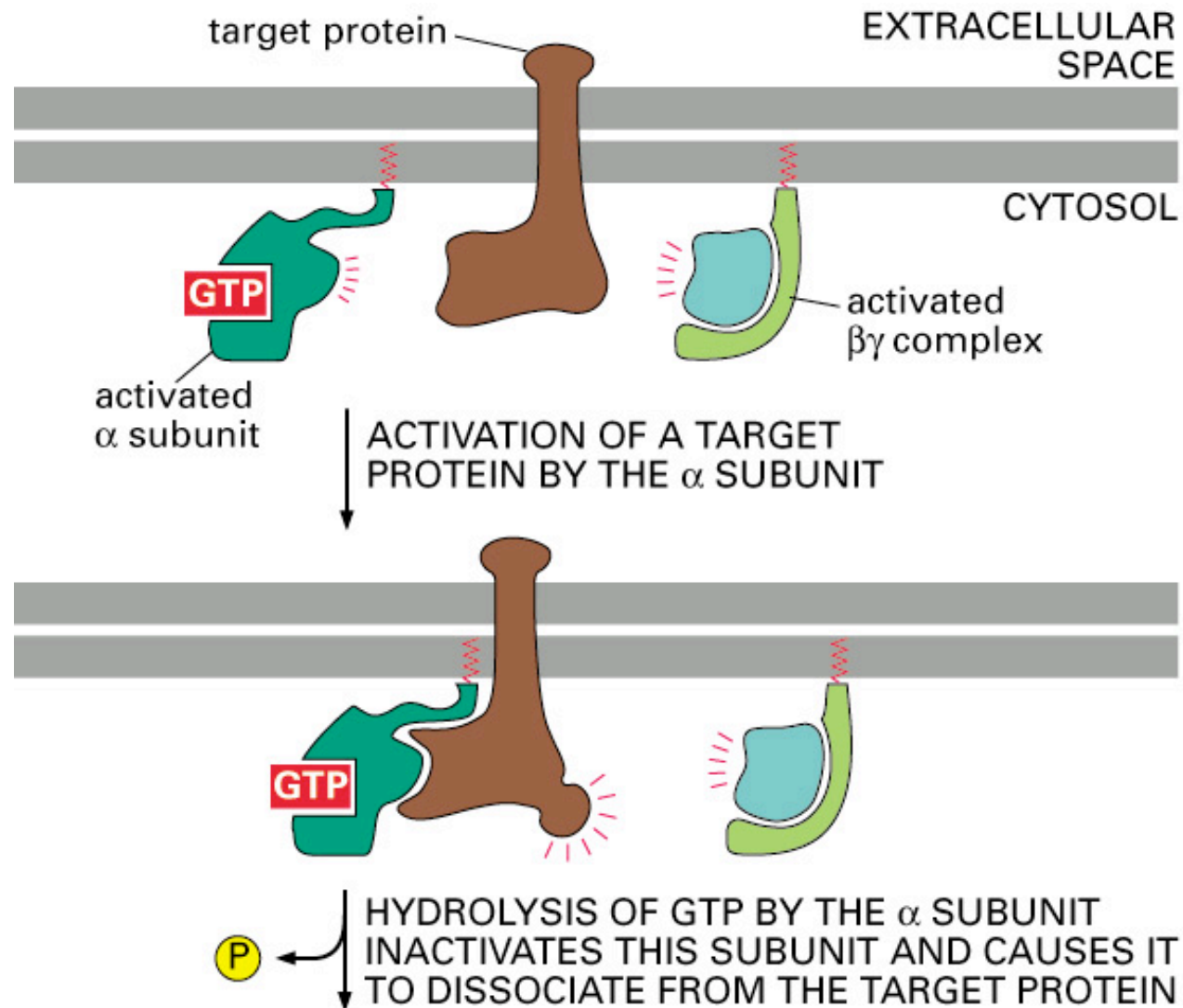


Figure 15-29 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

GTP hydrolysis ends signaling and induces trimerization

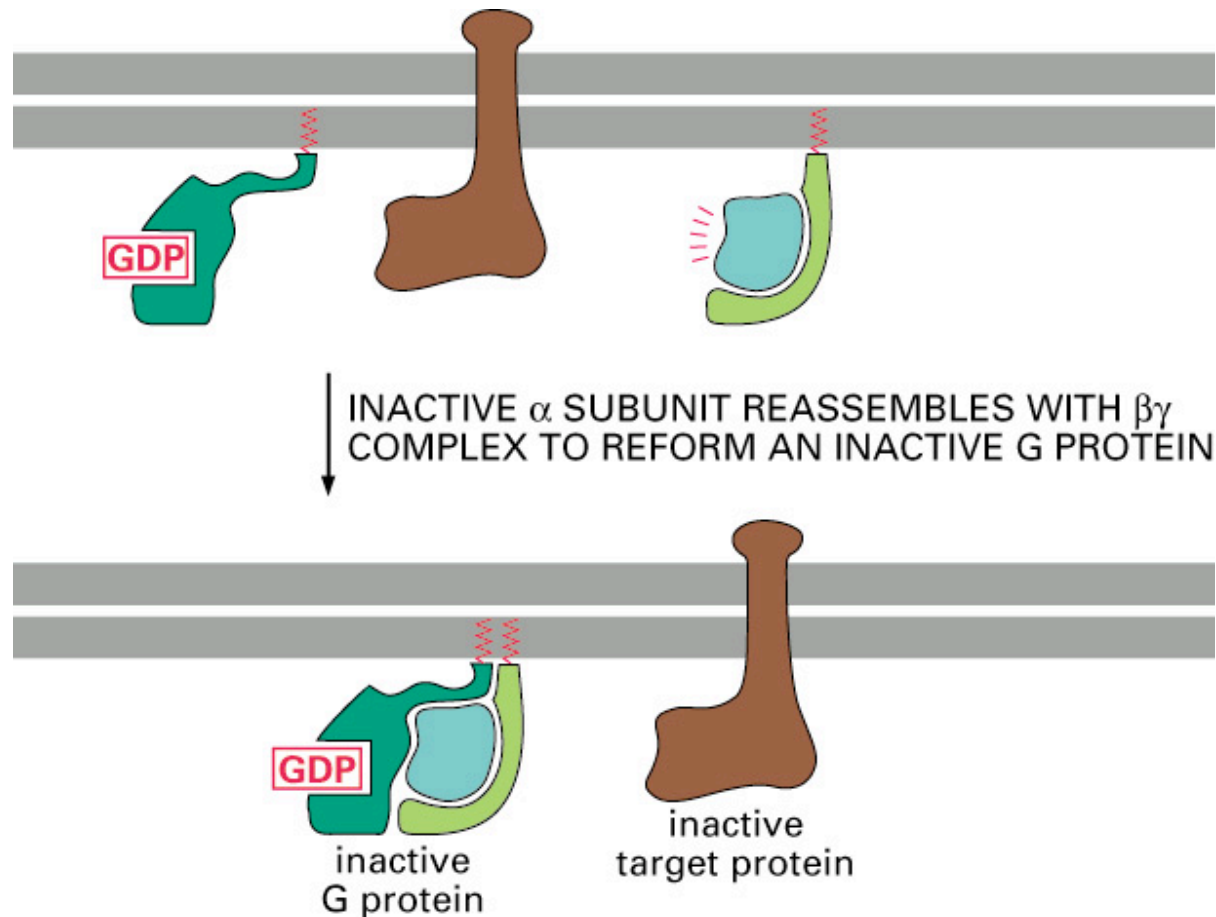
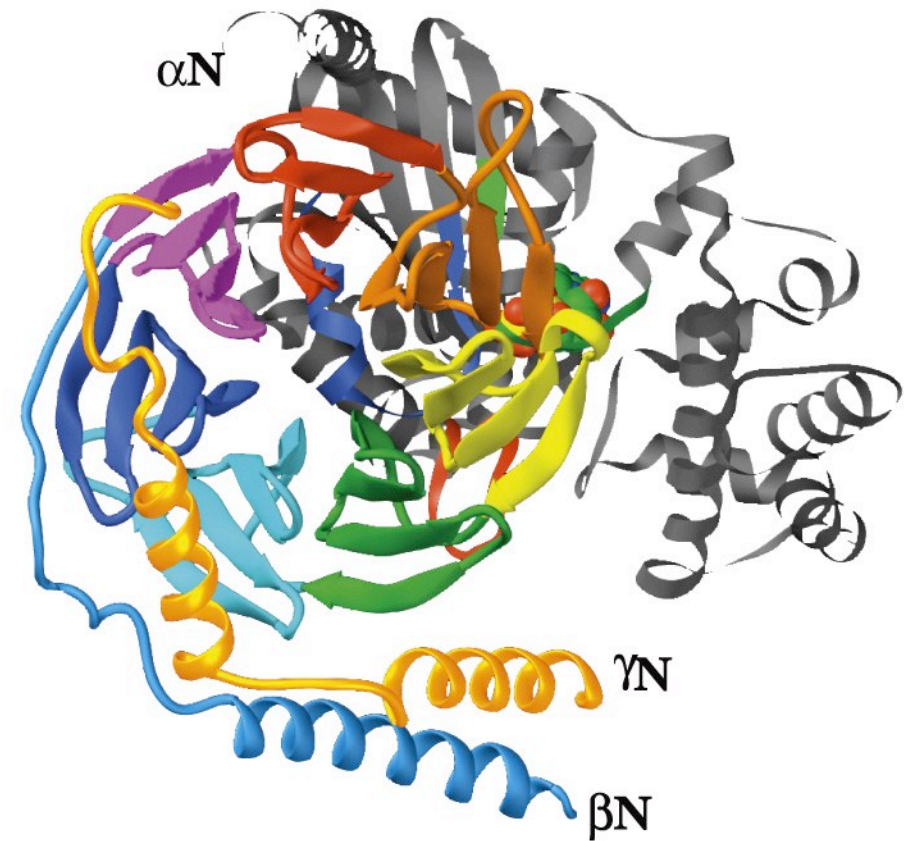
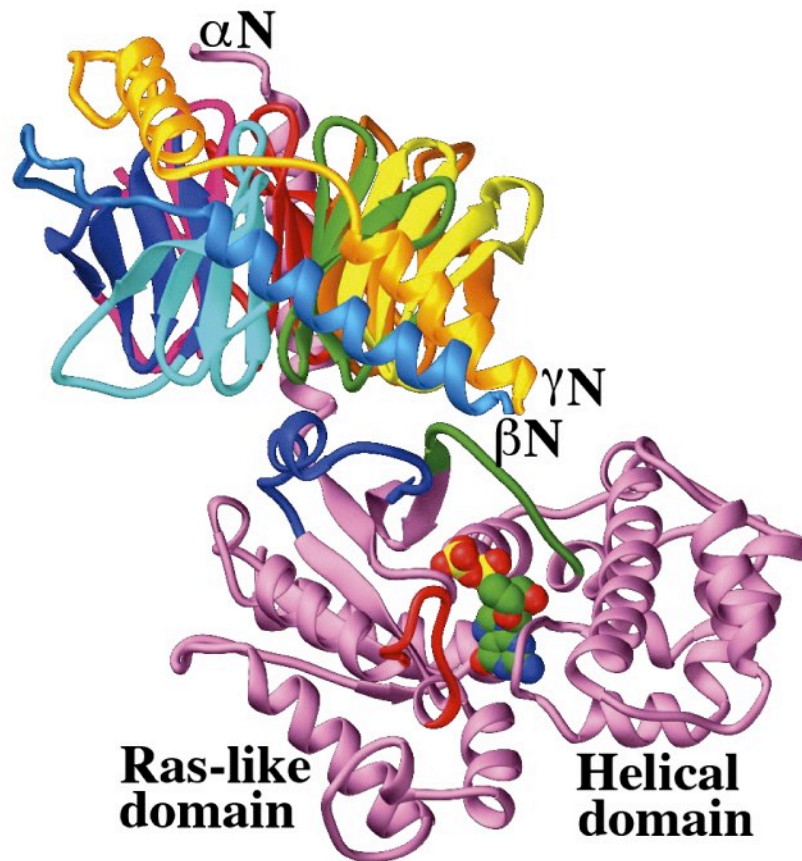


Figure 15–29 part 2 of 2. Molecular Biology of the Cell, 4th Edition.



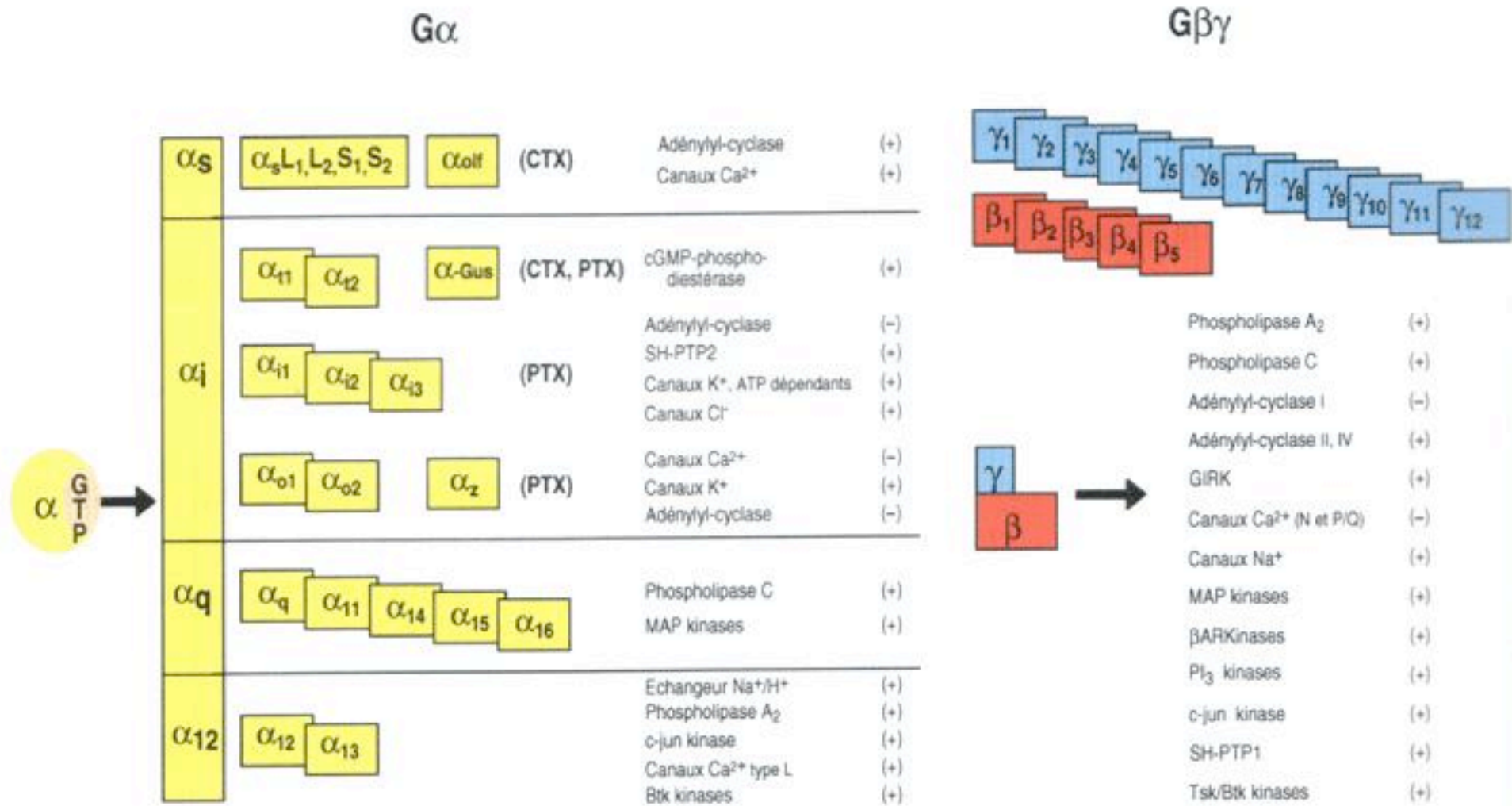
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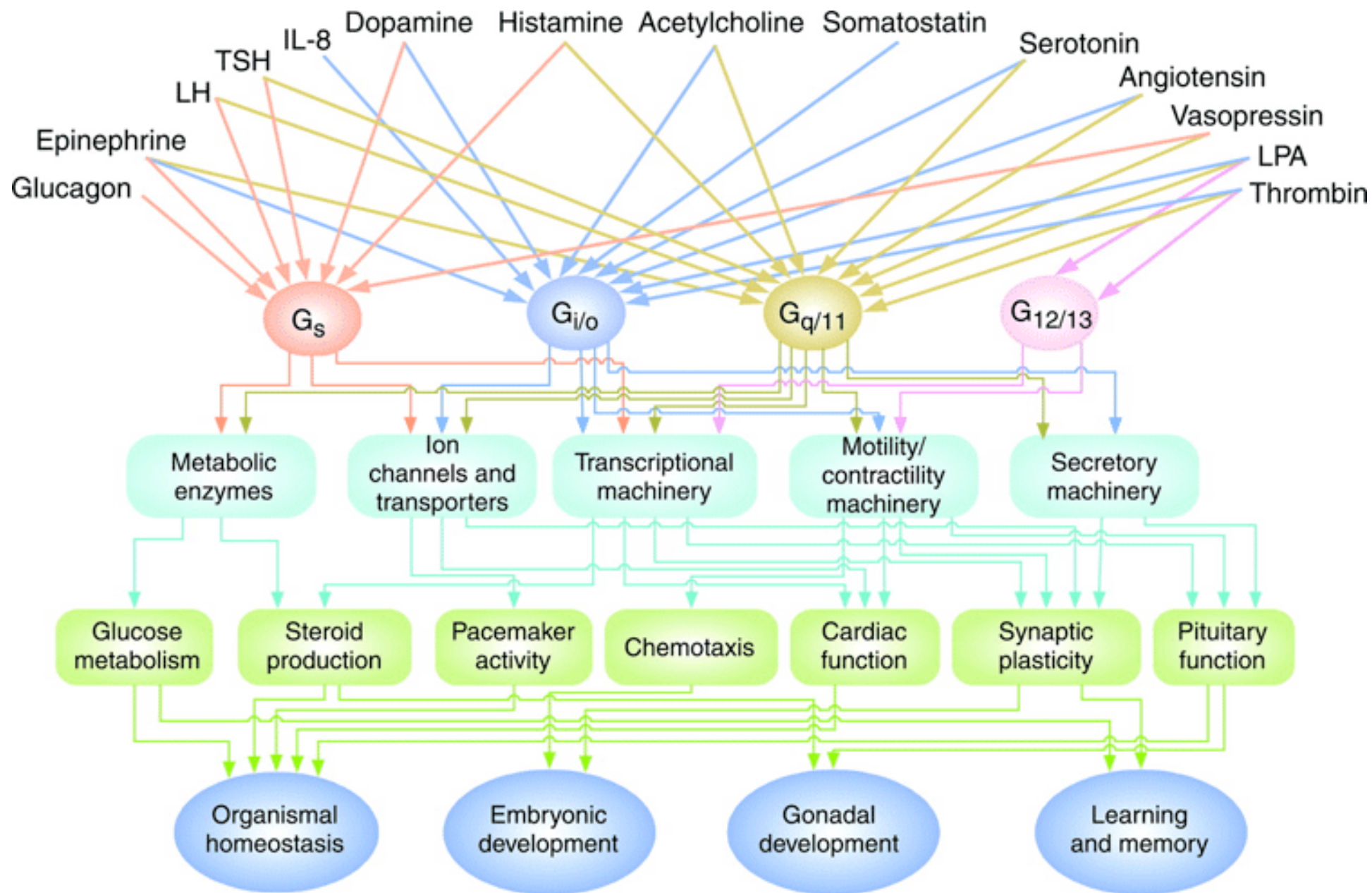
**Figure 13-19 Protéine-G trimérique -
sous unités alpha/beta/gamma**

Variety of G-proteins

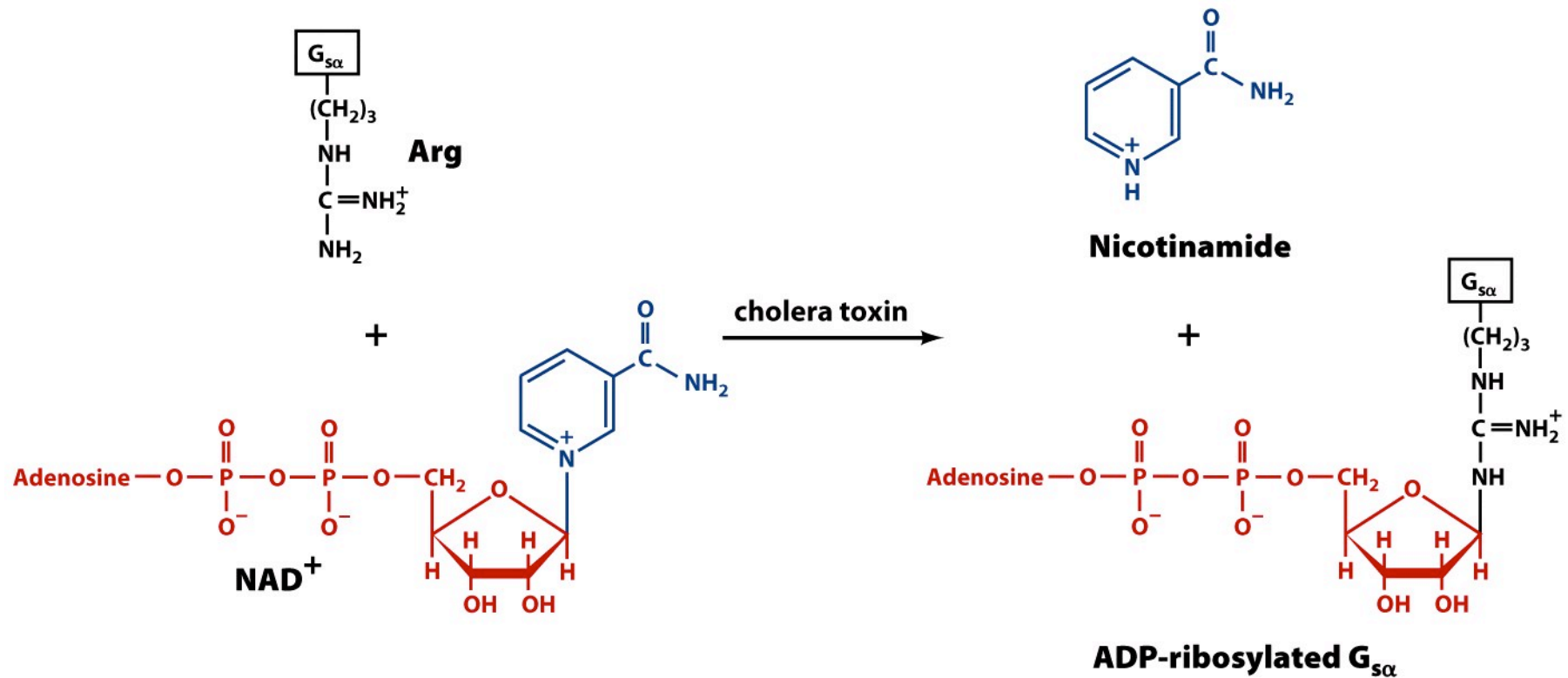
- G_s are stimulatory
 - G_i/o are inhibitory
 - G_q act on PLC
 - $G_{12/13}$ act on ion channels
-
- 22 α subunits
 - 5 β subunits
 - 12 γ subunits

VARIETY OF G-PROTEINS: $G\alpha$, $- \beta$, $- \gamma$





ADP-ribosylation



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par cholera toxine de $G_{s\alpha}$
 par pertussis toxine de $G_{i\alpha}$

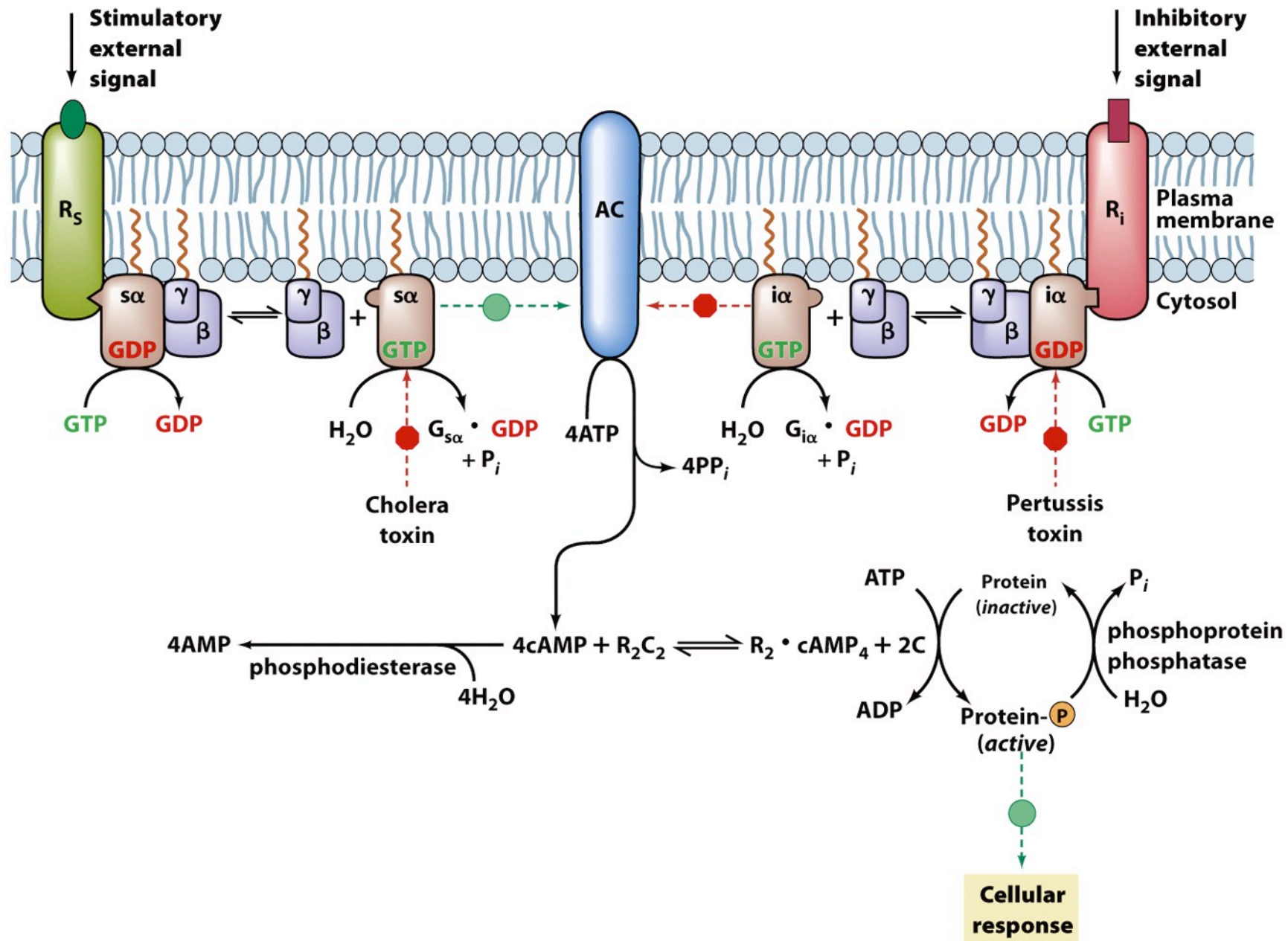
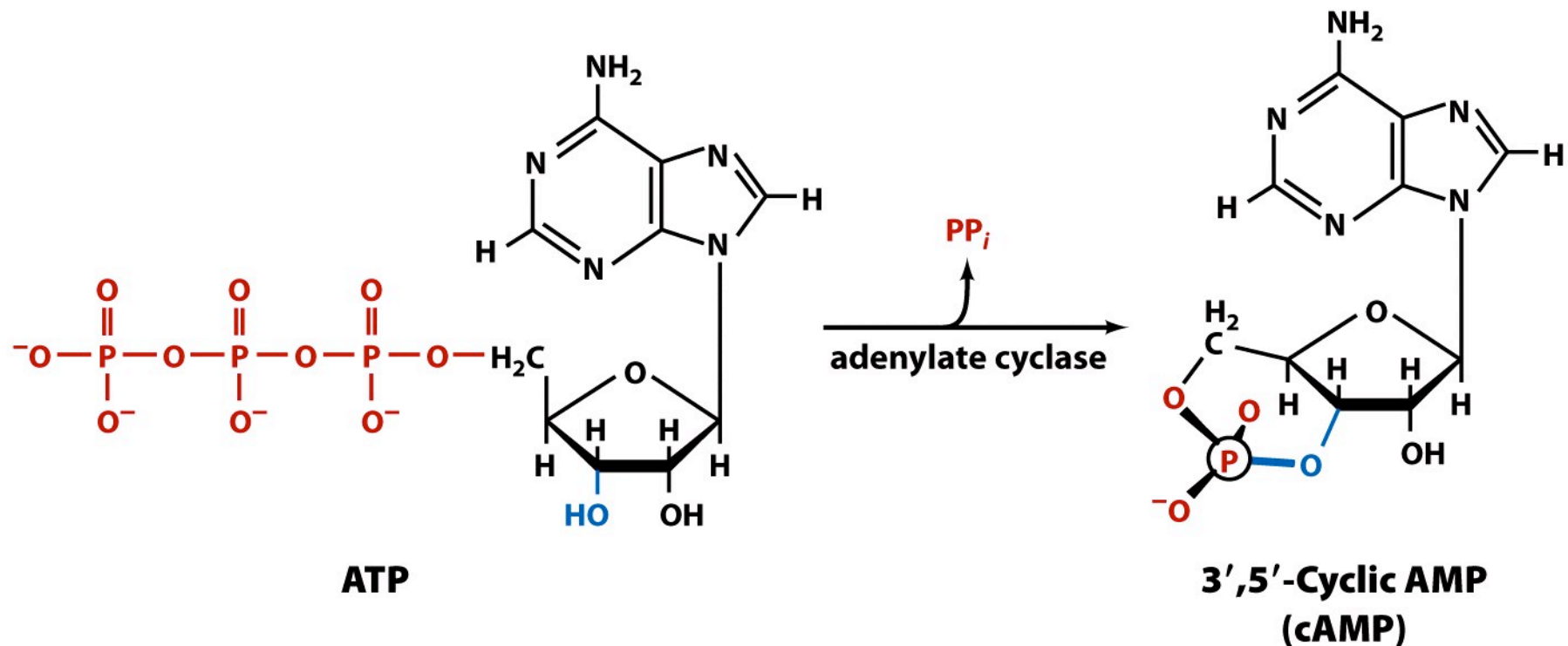


Figure 13-23 Système Adenylate Cyclase

Heterotrimeric G-Proteins

The cAMP cascade



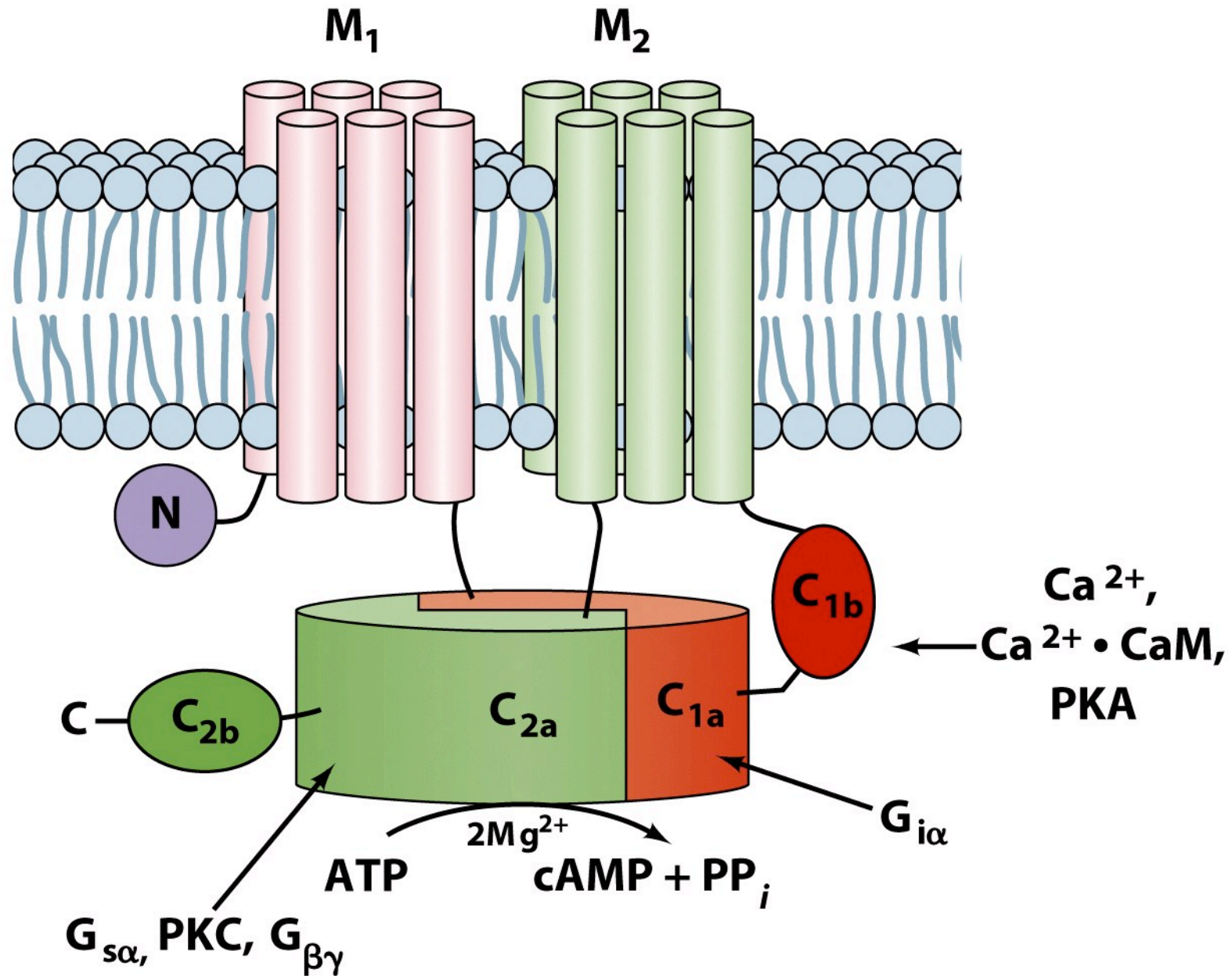
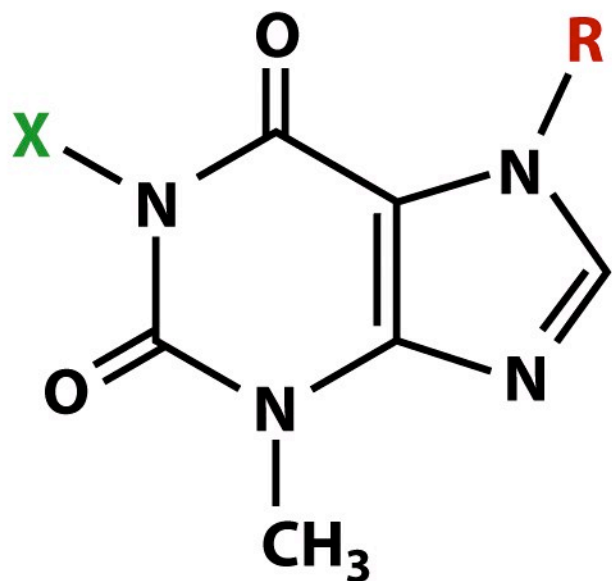


Figure 13-20 Structure de Adenylate Cyclase -
M1/M2: =6TM / C1a/C2a:= domaines catalytiques pseudosymétriques



R = CH₃

X = CH₃

Caffeine (1,3,7-trimethylxanthine)

R = H

X = CH₃

Theophylline (1,3-dimethylxanthine)

R = CH₃

X = H

Theobromine (1,7-dimethylxanthine)

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PKA activation by cAMP

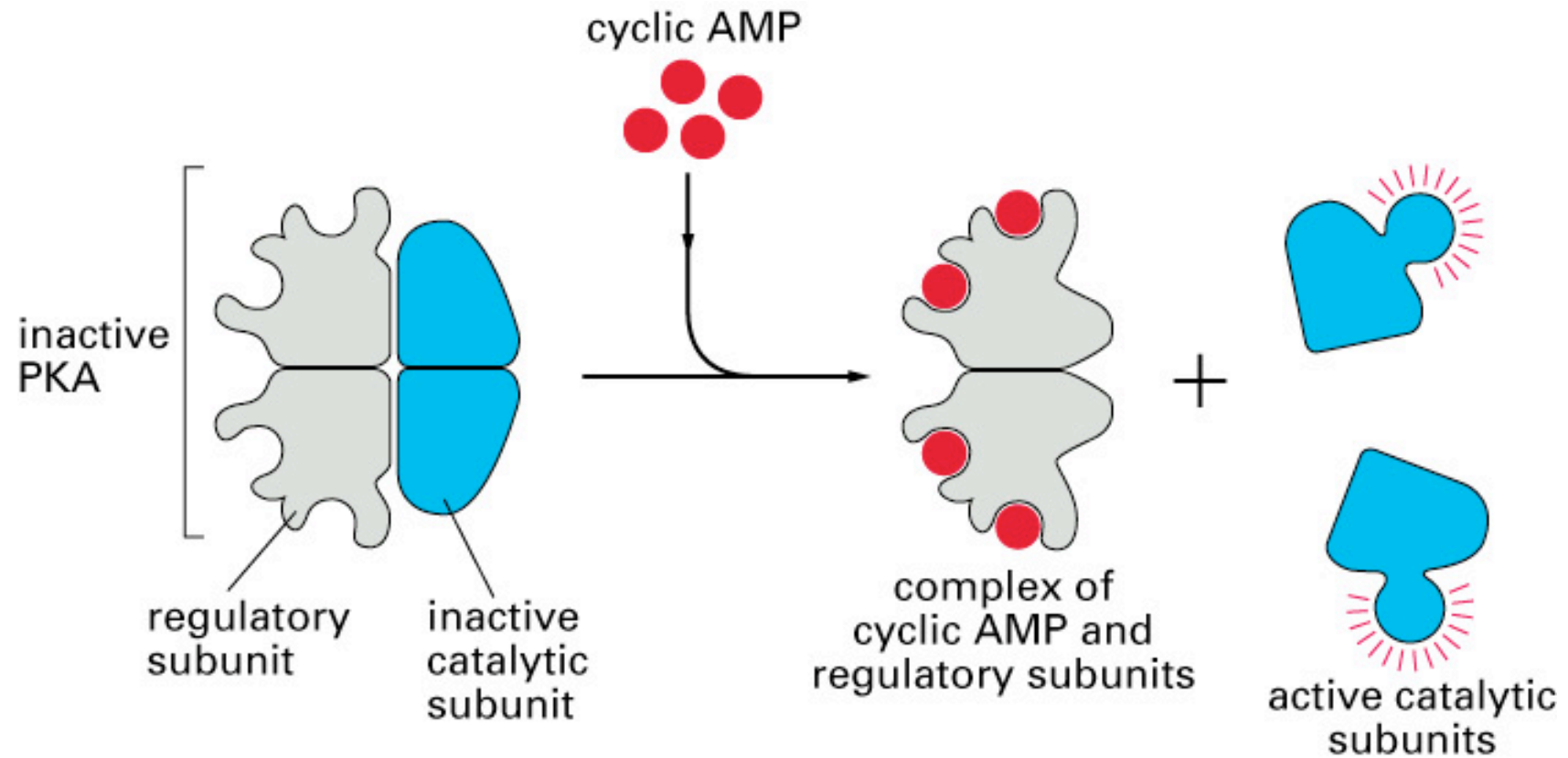


Figure 15–32. Molecular Biology of the Cell, 4th Edition.

PKA activates gene expression

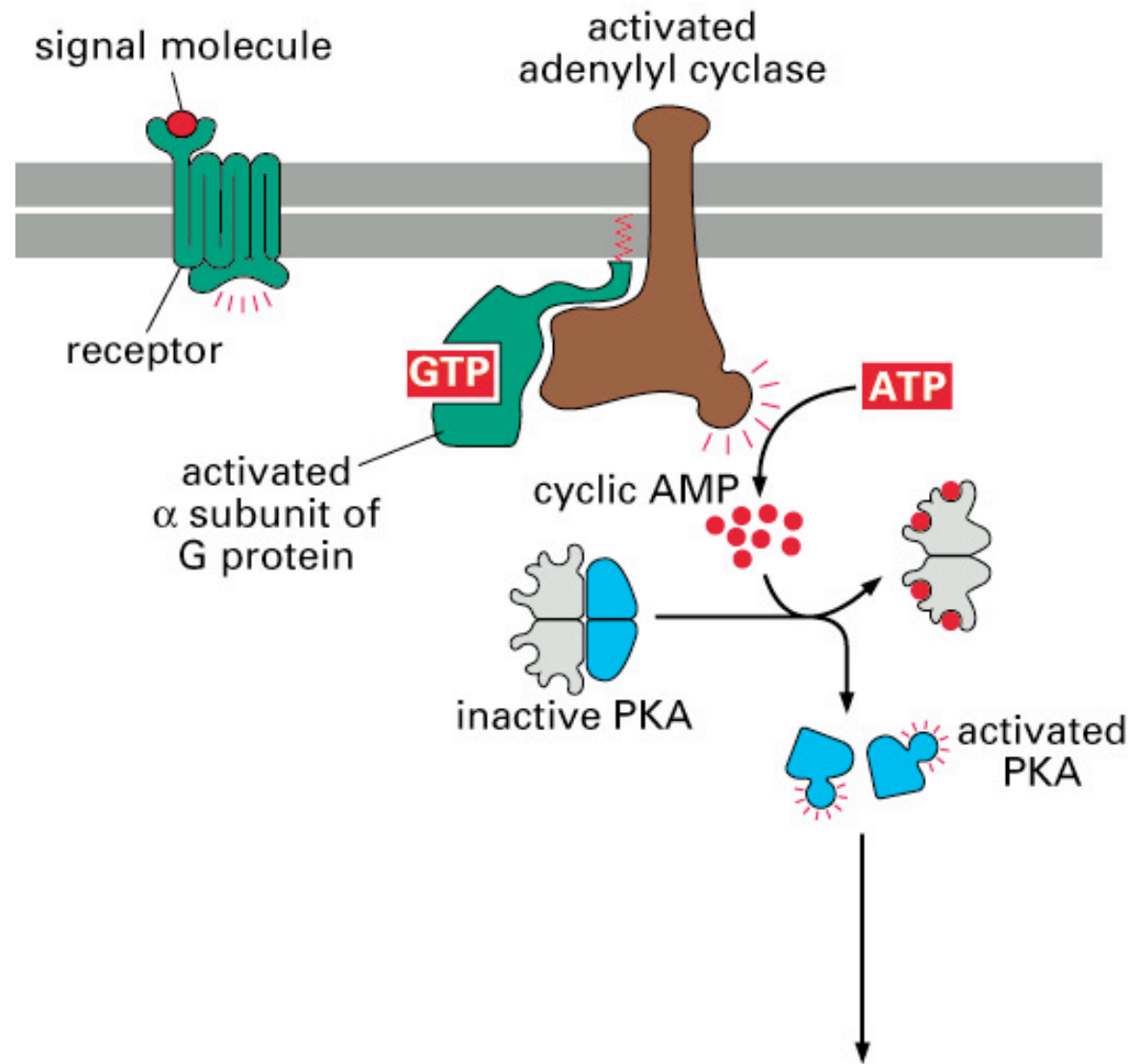
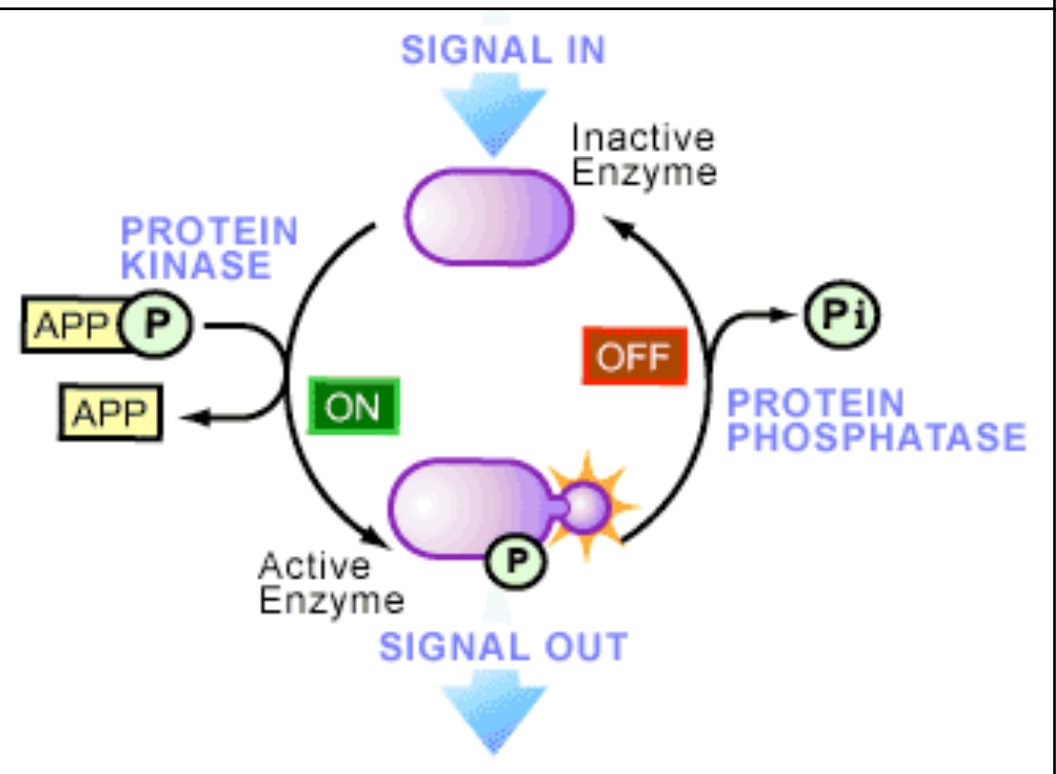
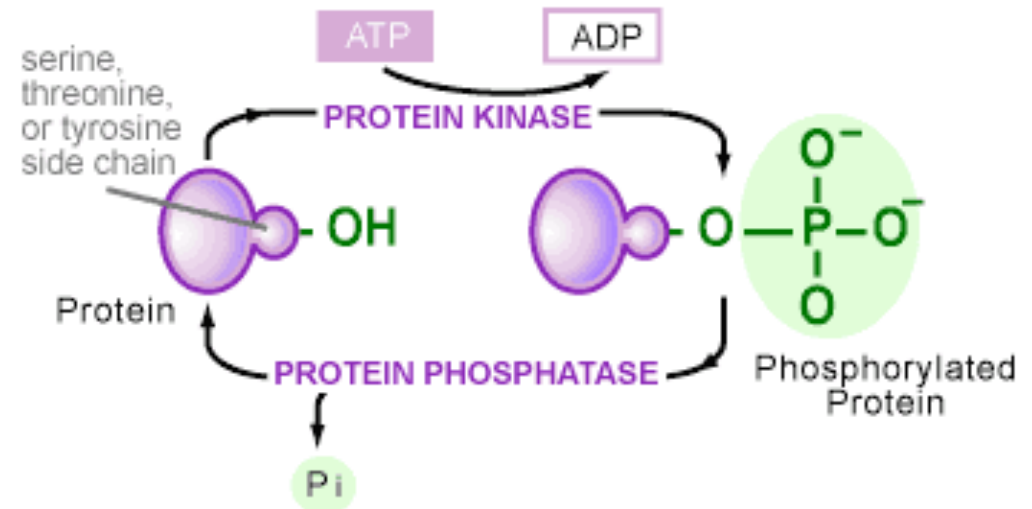
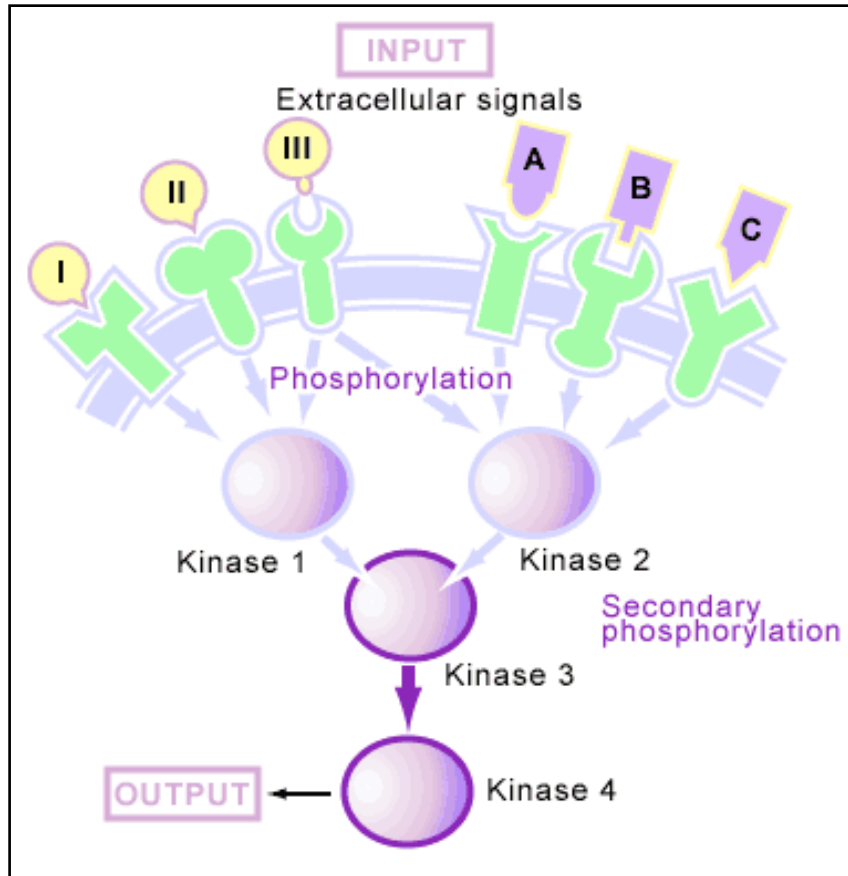
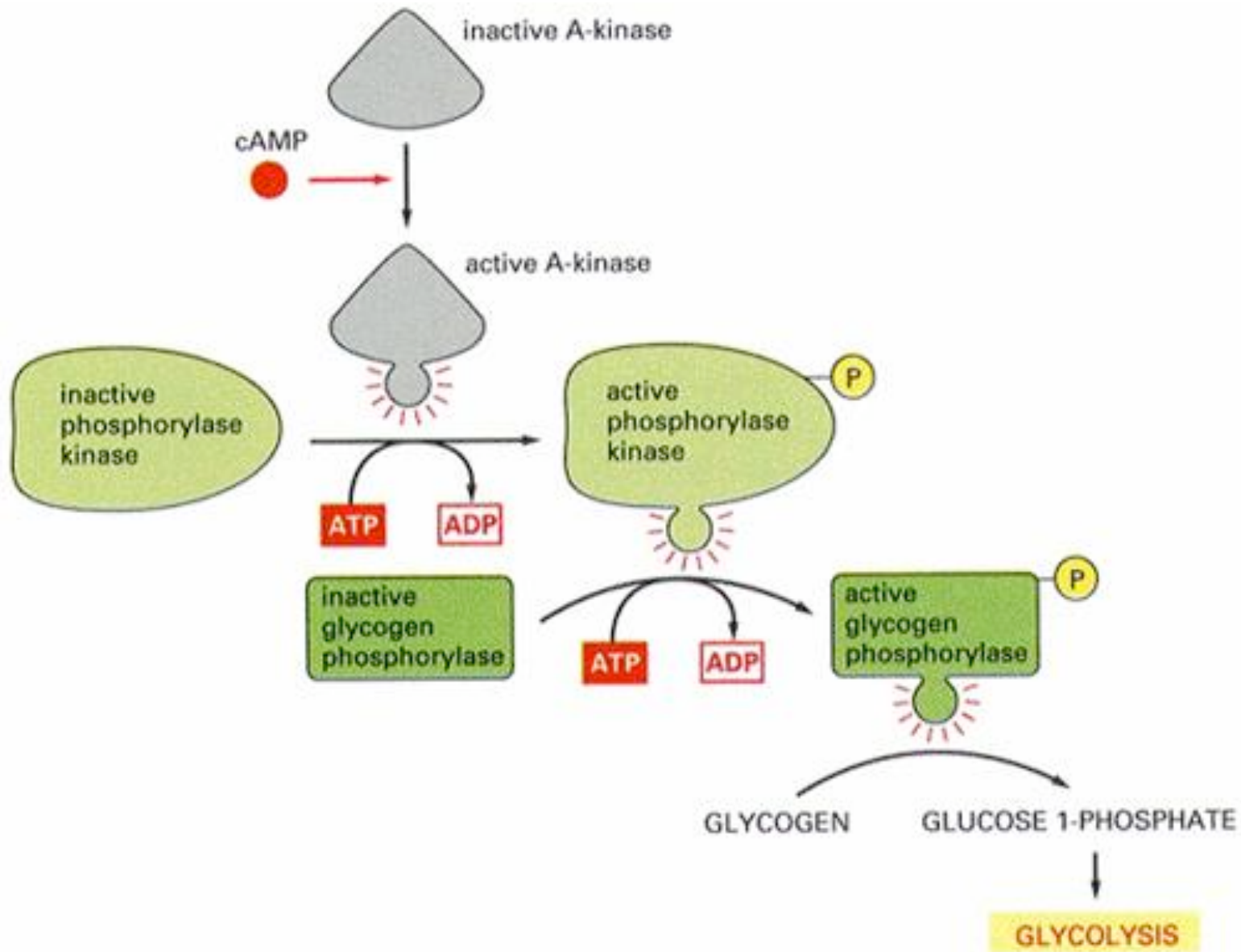


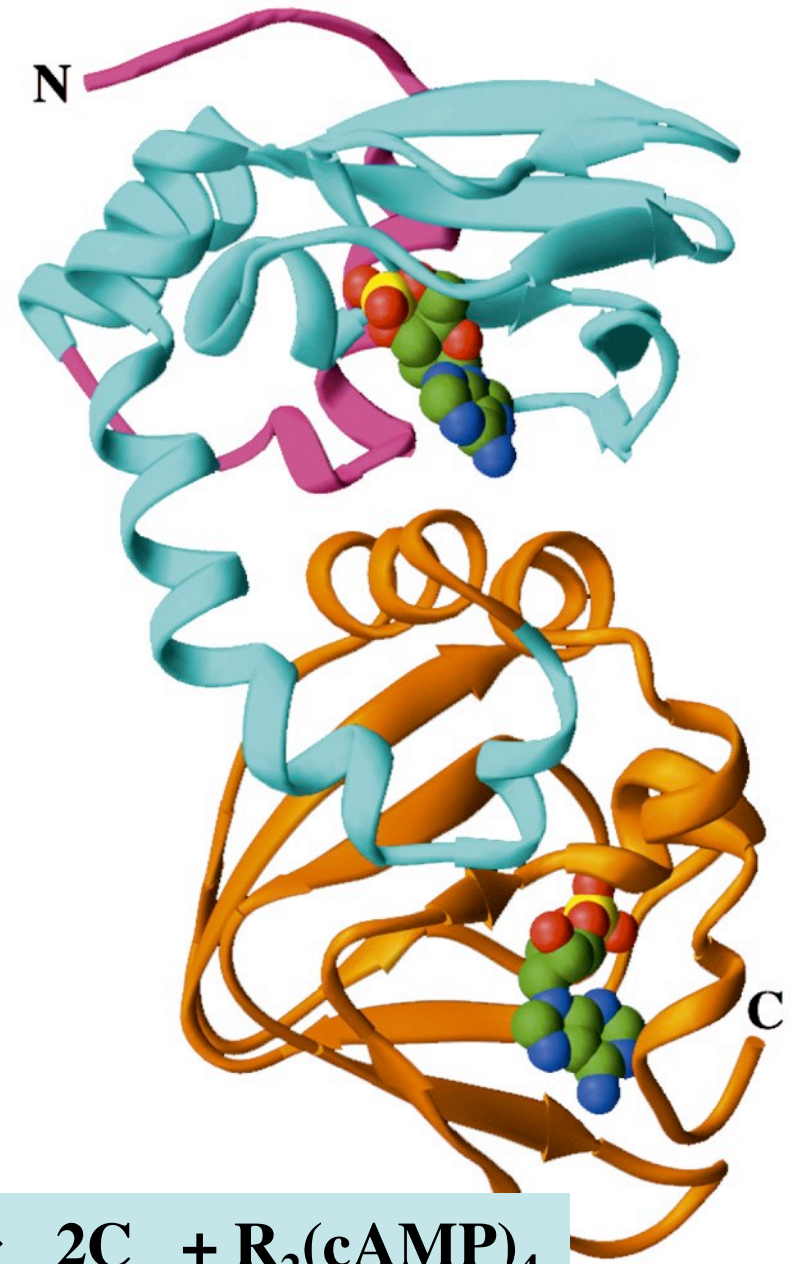
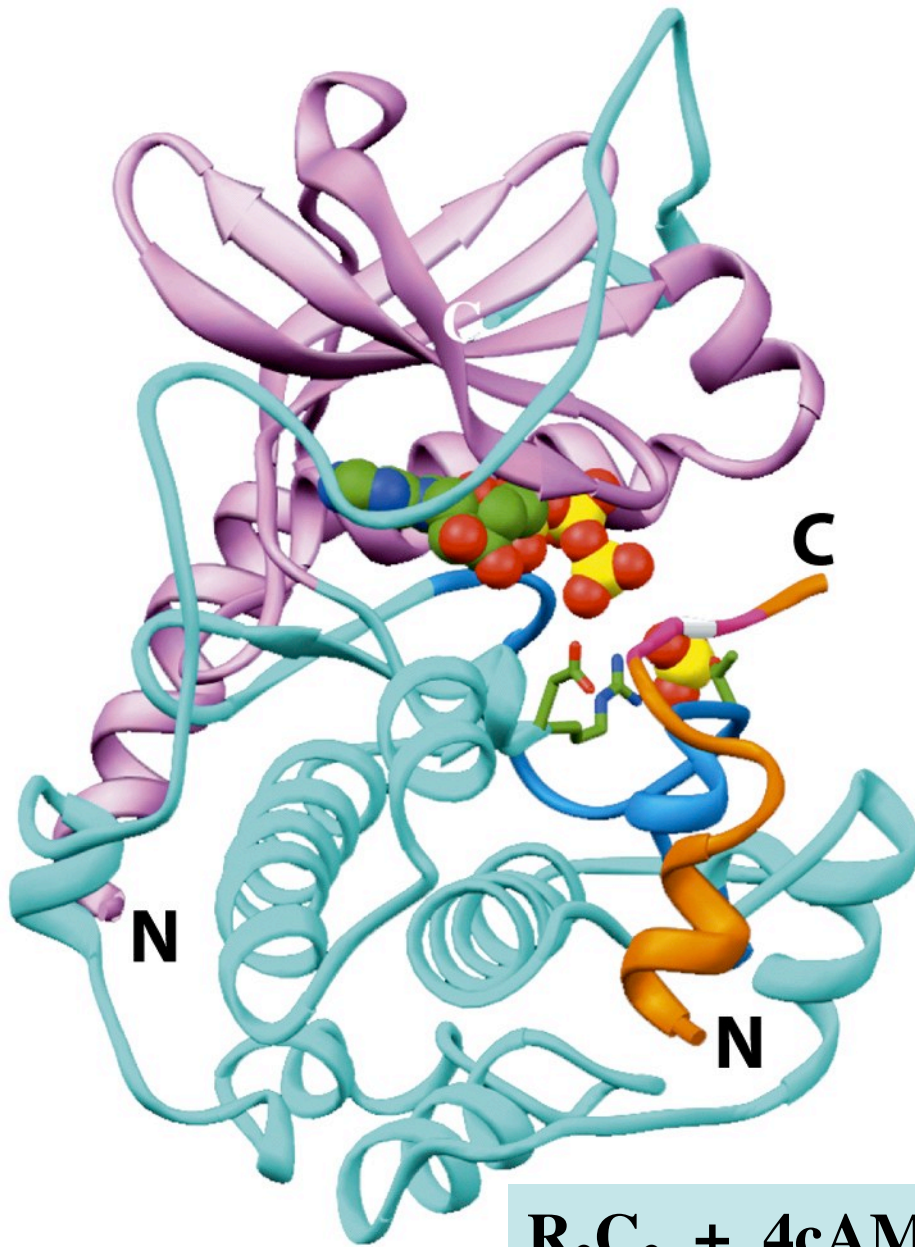
Figure 15-33 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

CASCADE de PHOSPHORYLATION



EXEMPLE: Métabolisme du Glycogène





Inactif

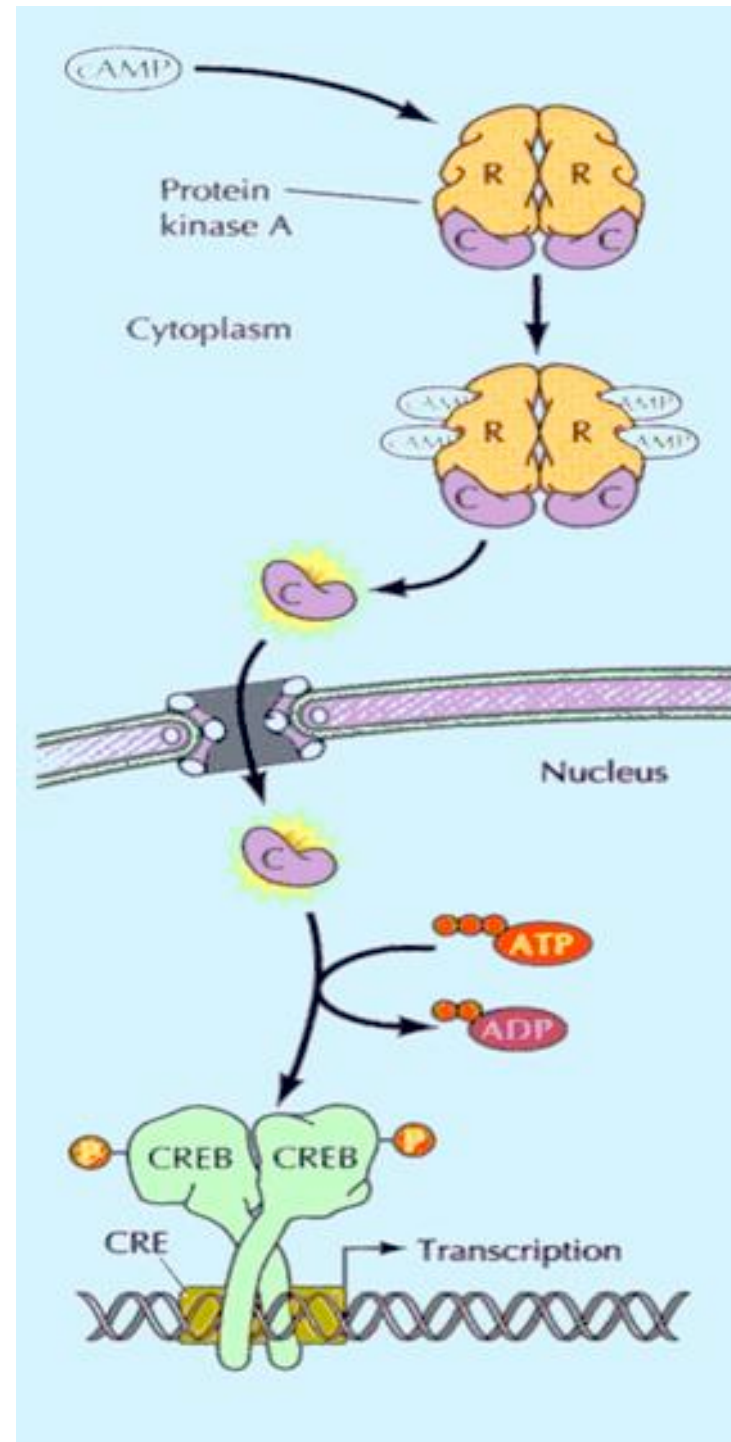
actif

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Figure 13-21 PKA : domaine catalytique

domaine régulateur

CREB = cAMP responsive Elements Binding Protein



Inactivation of PKA pathway

The G-protein -PKA pathway is inactivated by:

- Receptor desensitization (phosphorylation by **PKA**)
- GTP hydrolysis in G-protein (**GTPase** of α -subunit)
- cAMP hydrolysis by **phosphodiesterase**
- PKA inhibition
- Phosphatase** action on PKA targets
- Activation of an antagonistic pathway (G_i)

Turn off of the signal:

1. G_{α} hydrolyzes GTP to GDP + P_i . (**GTPase**).

The presence of **GDP** on G_{α} causes it to rebind to the inhibitory **$\beta\gamma$** complex.

Adenylate Cyclase is no longer activated.

2. **Phosphodiesterase** catalyzes hydrolysis of **cAMP $\rightarrow$ AMP**.

Turn off of the signal (cont.):

3. Hormone Receptor **desensitization** occurs.
This process varies with the hormone.

- ◆ Some receptors are **phosphorylated** via G-protein-coupled receptor kinases.
- ◆ The phosphorylated receptor may then bind to a protein **arrestin** that blocks receptor-G-protein activation & promotes removal of the receptor from the membrane by clathrin-mediated endocytosis.

4. **Protein Phosphatase** catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A.

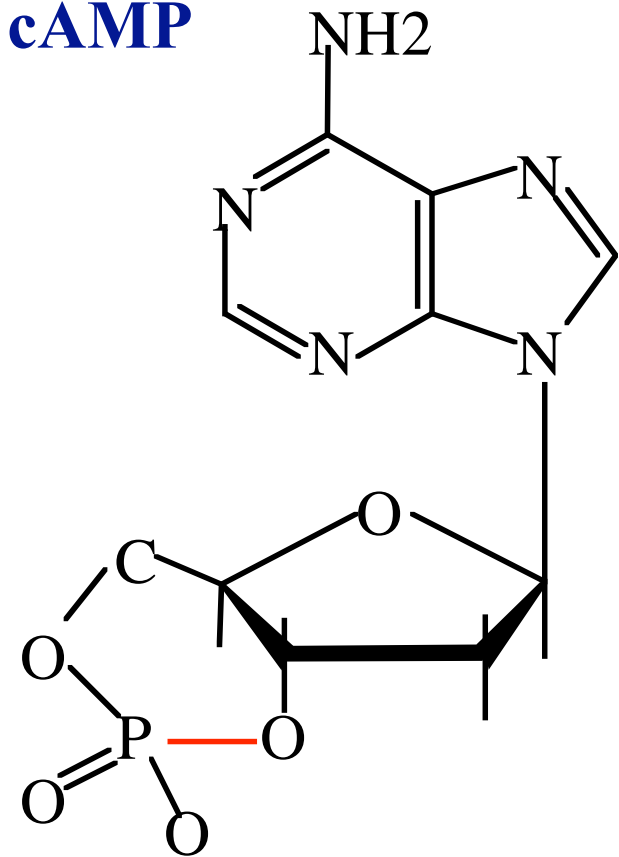
Phosphodiesterase enzymes catalyze:



The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.

Thus **cAMP stimulates its own degradation**, leading to rapid turnoff of a cAMP signal.

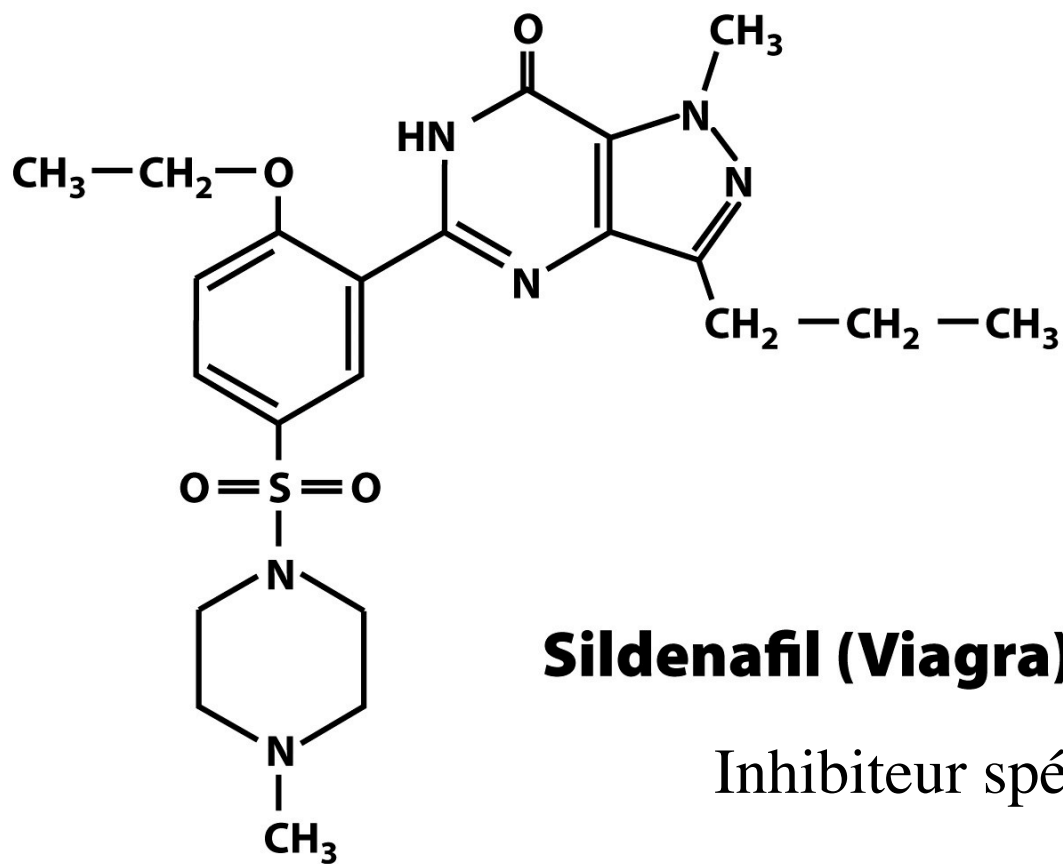
cAMP



Inhibiteur de Phosphodiesterase



PDE



Sildenafil (Viagra)

Inhibiteur spécifique de PDE5

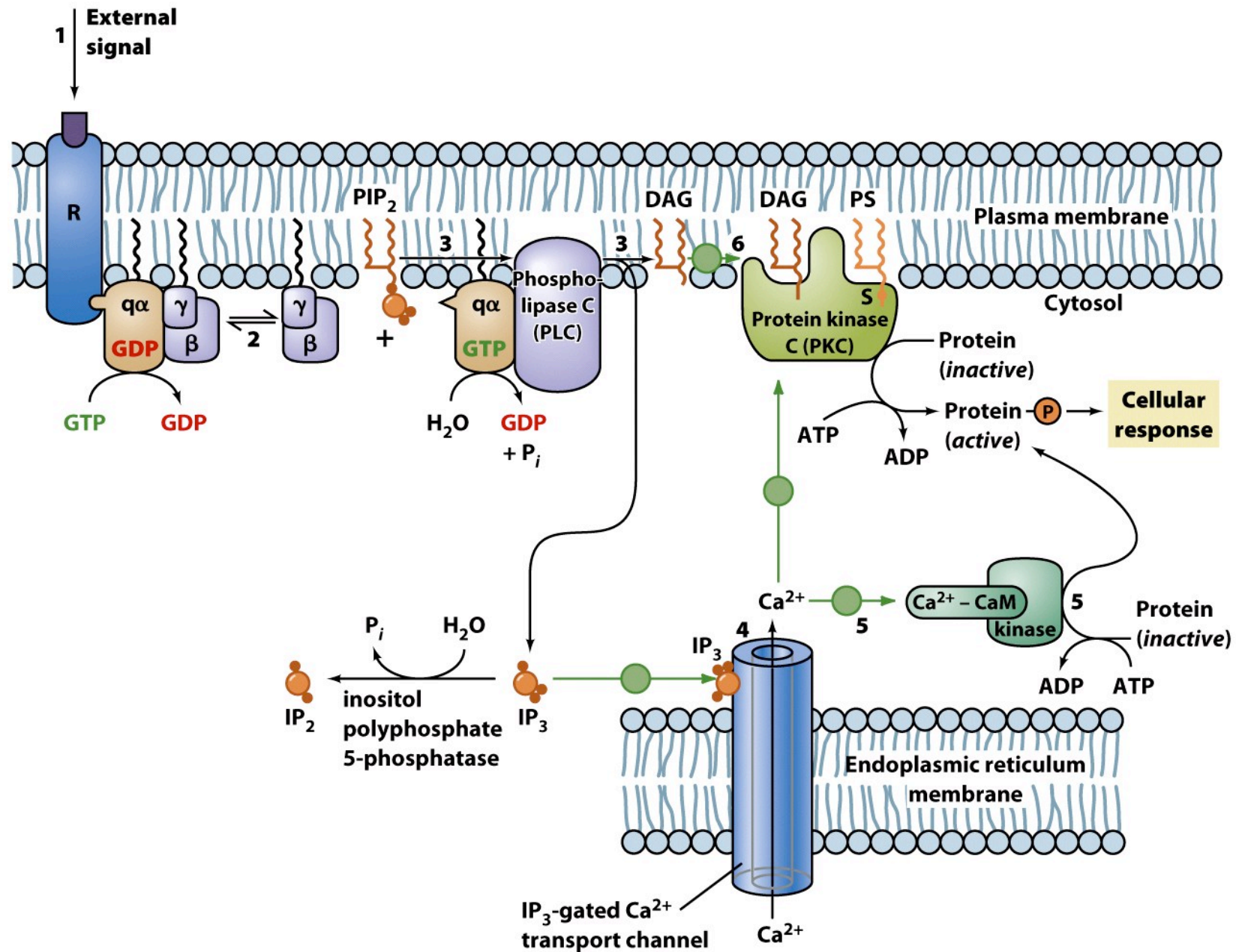
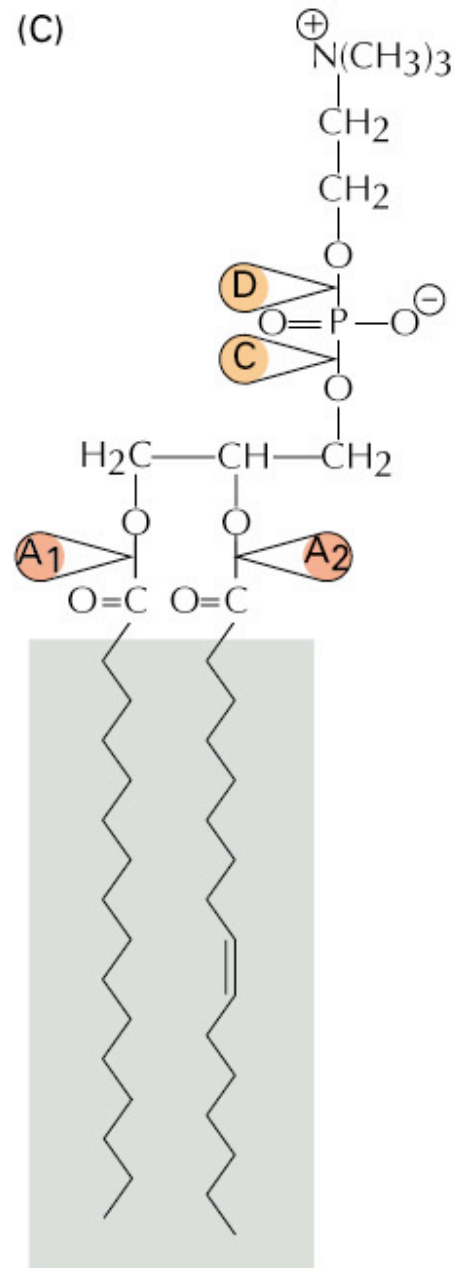


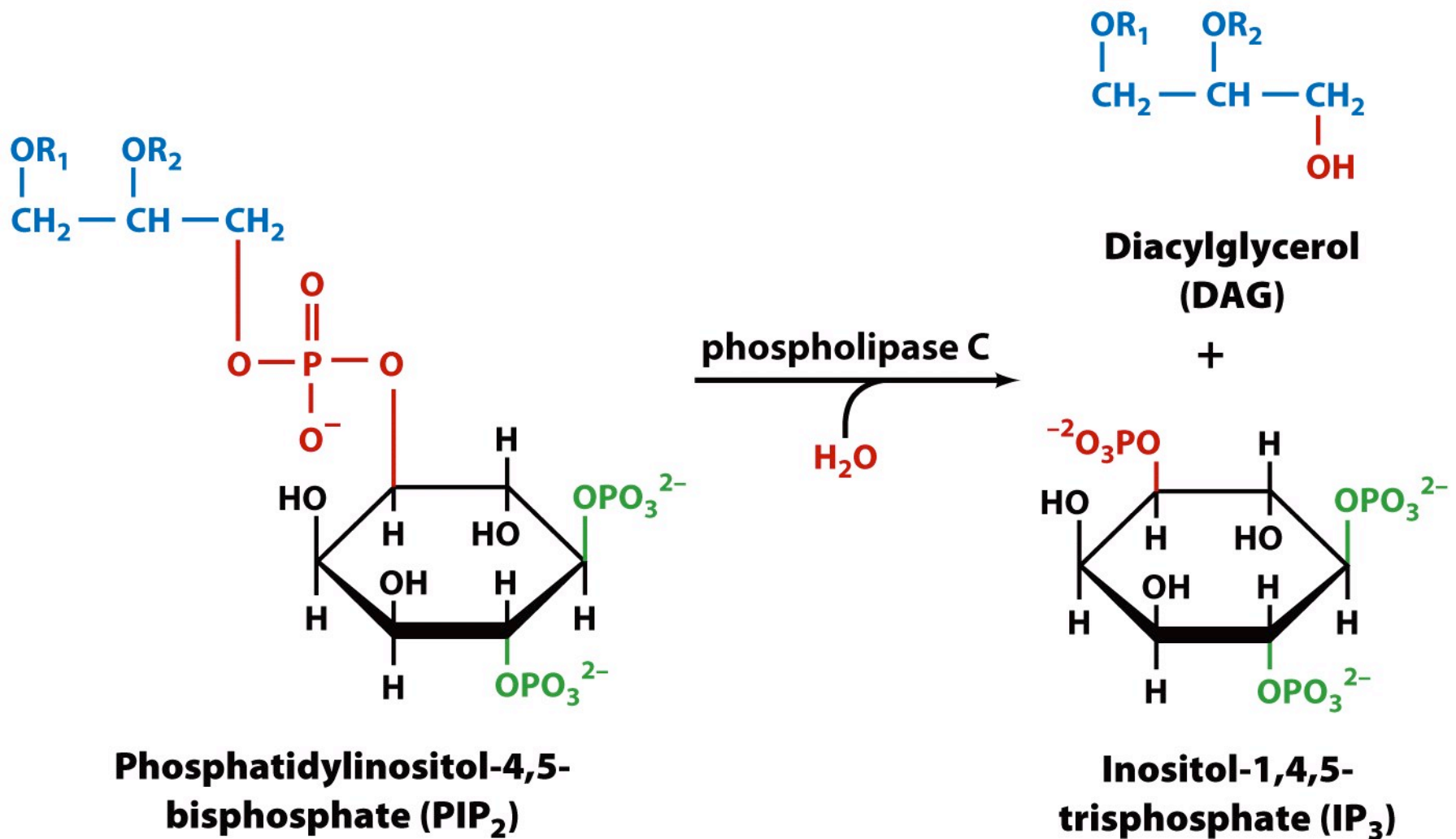
Figure 13-24 Système IP₃-DAG

(C)



Phospholipases

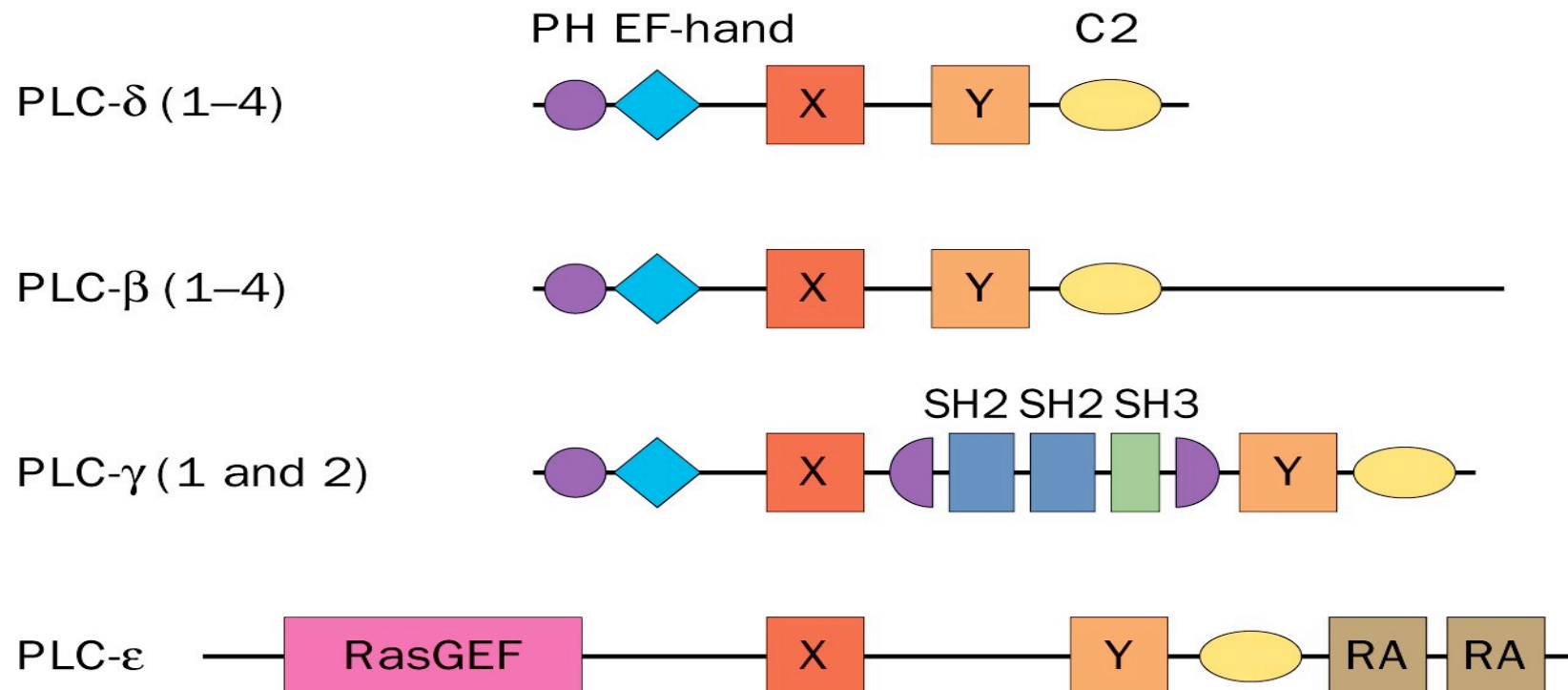
PLC generates DAG and
phosphoinositides, such as IP3
(inositol 1, 4, 5- triphosphate)

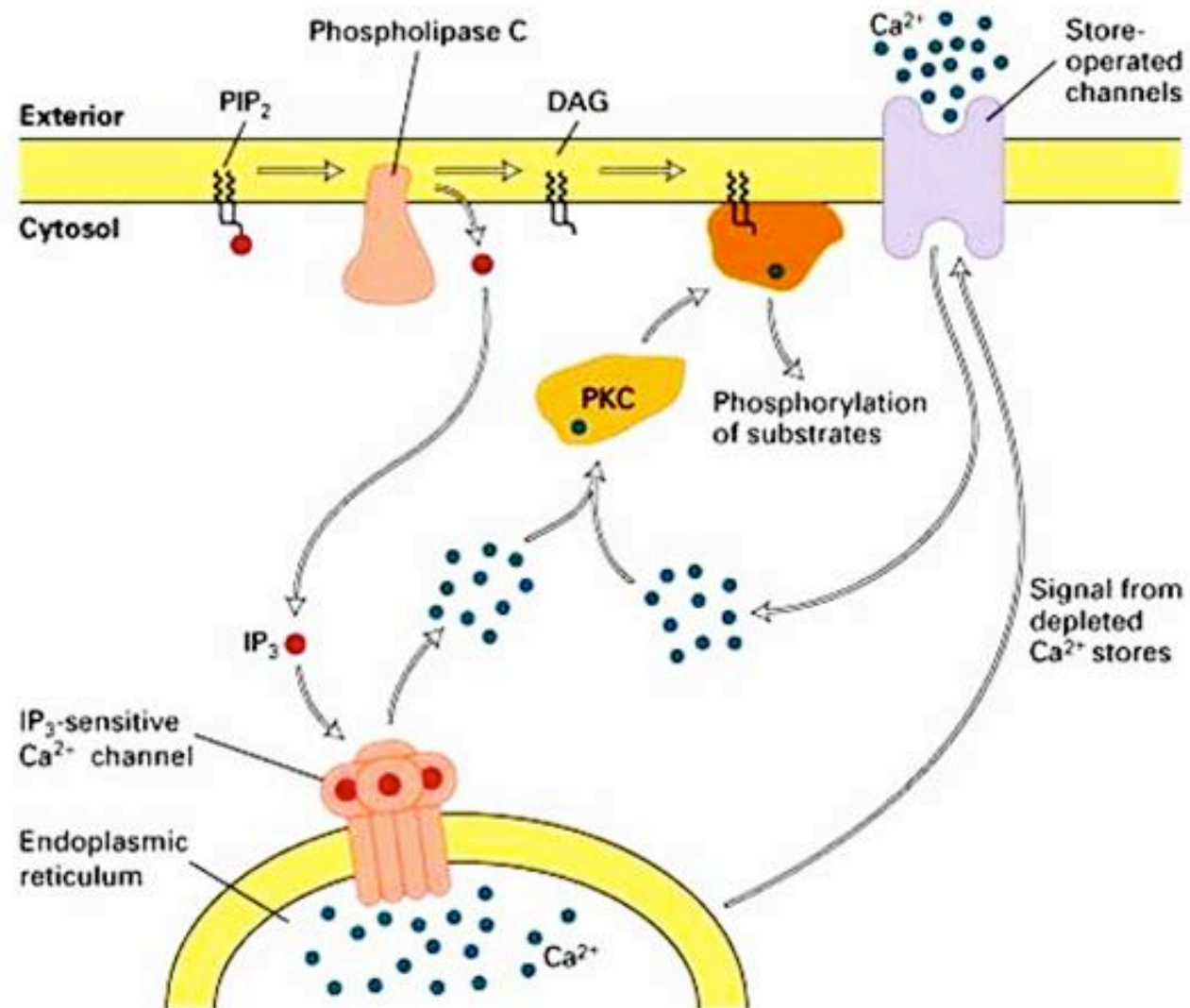


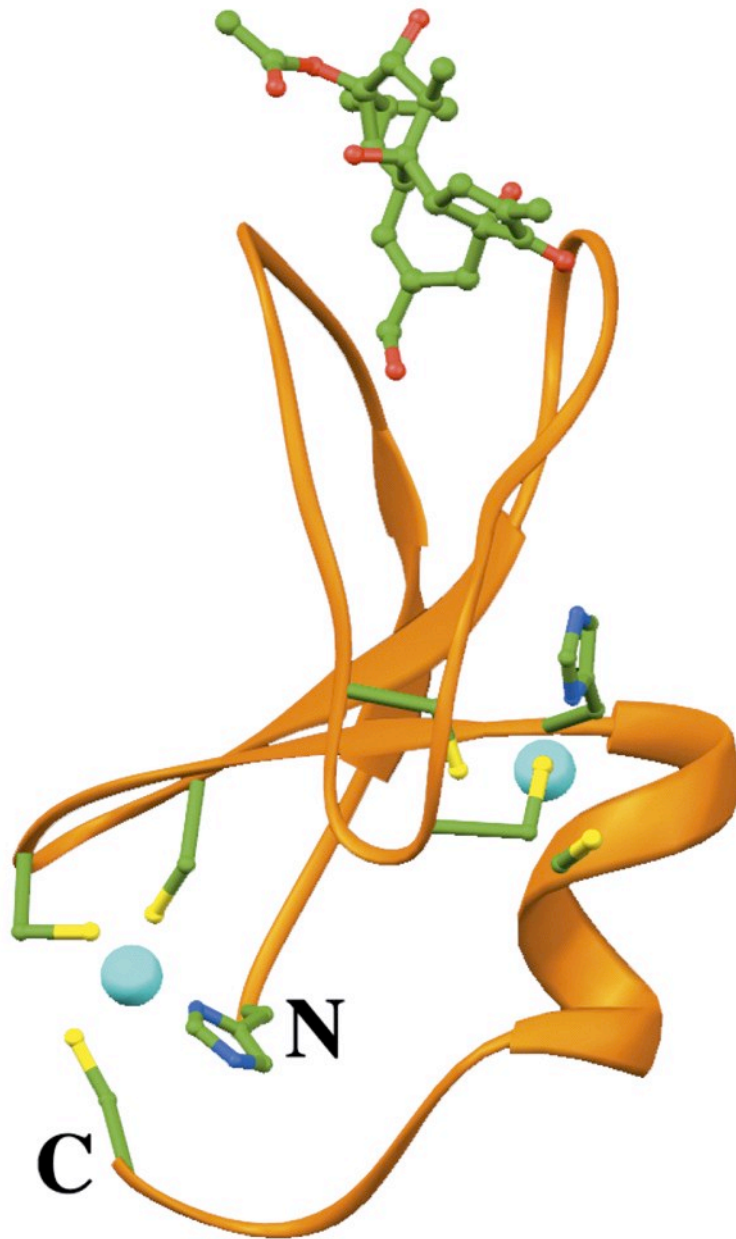
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Figure 13-25

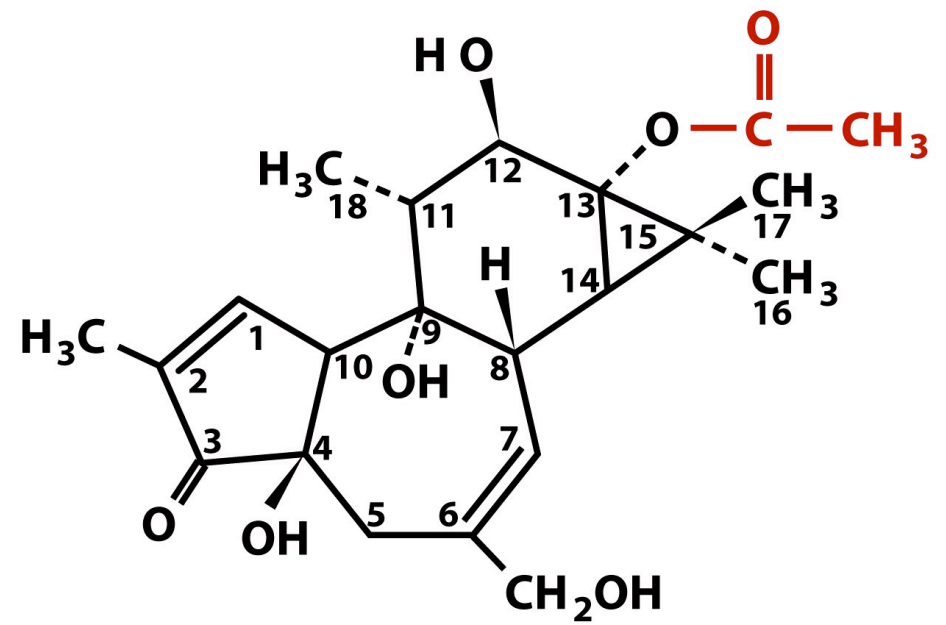
Domain organization of the four classes of phosphoinositide-specific PLCs.







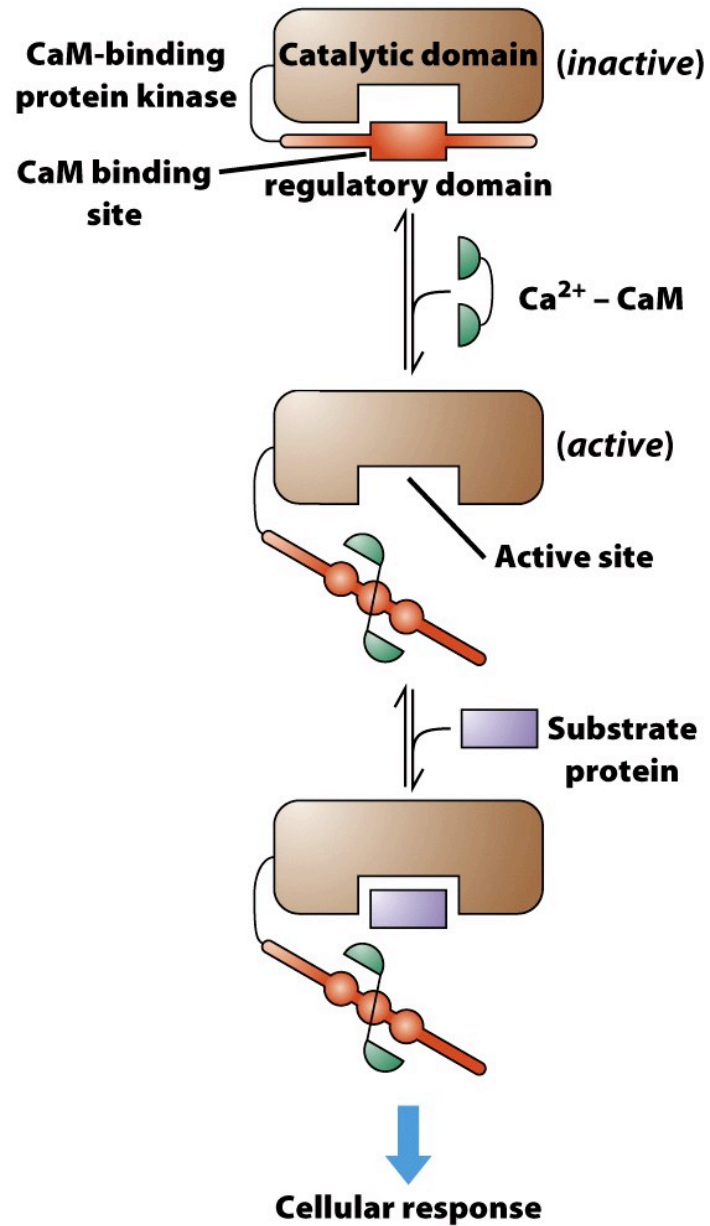
PKC



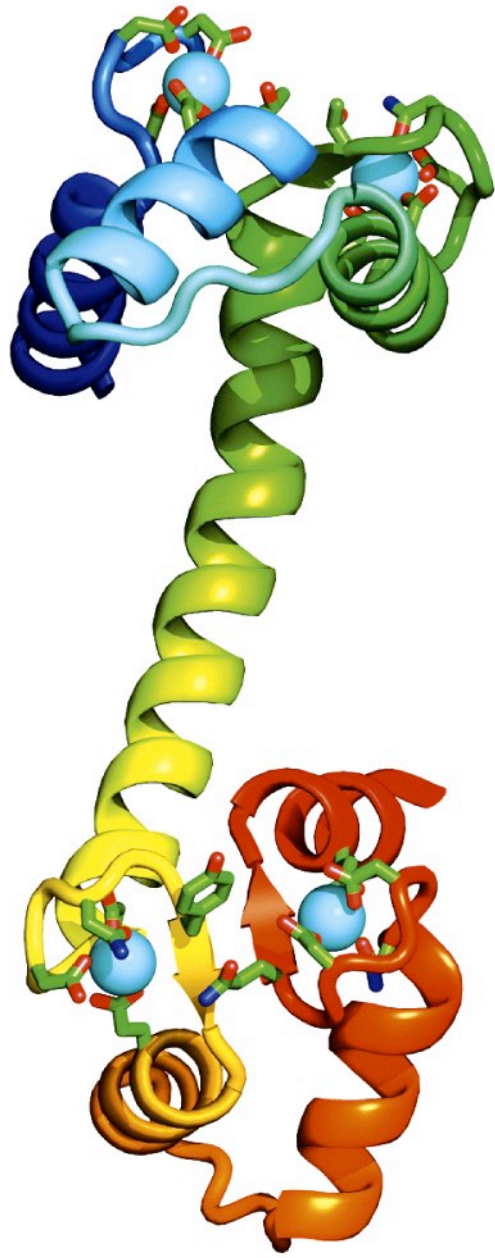
Phorbol-13-acetate

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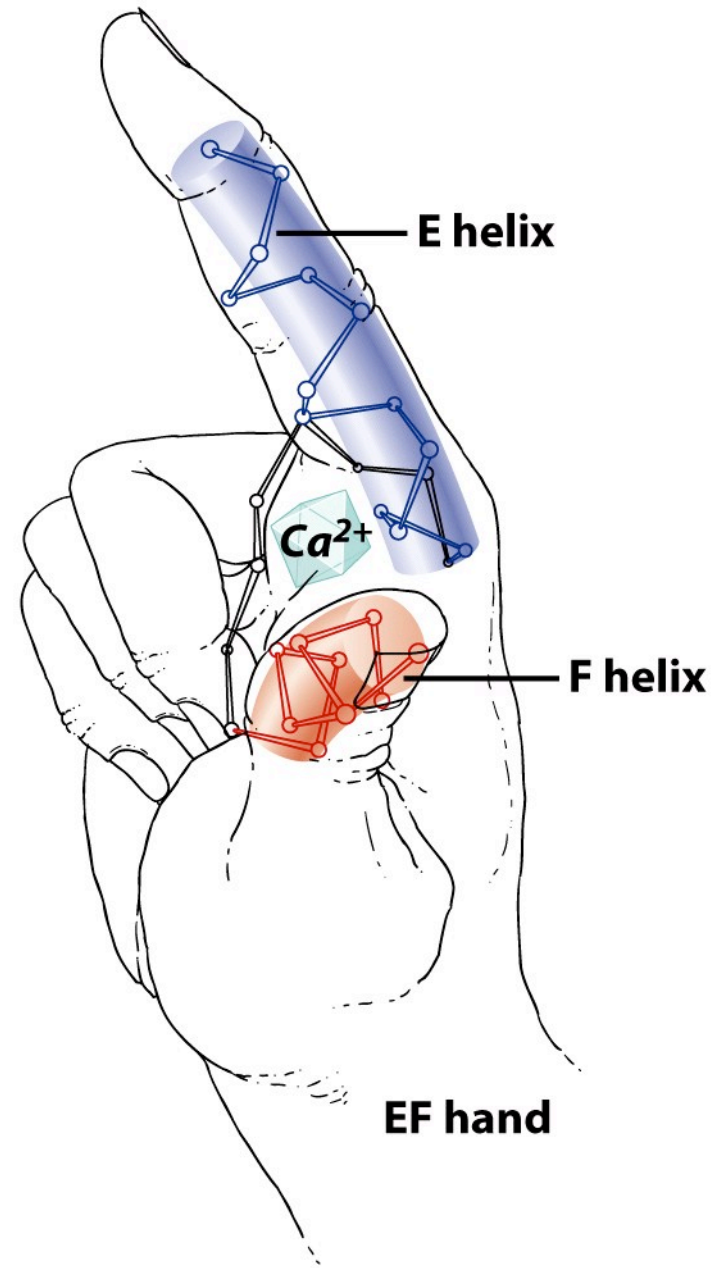
Inhibiteur de PKC



Role du Calcium: Calcium-calmoduline Protéine Kinase



Calmoduline

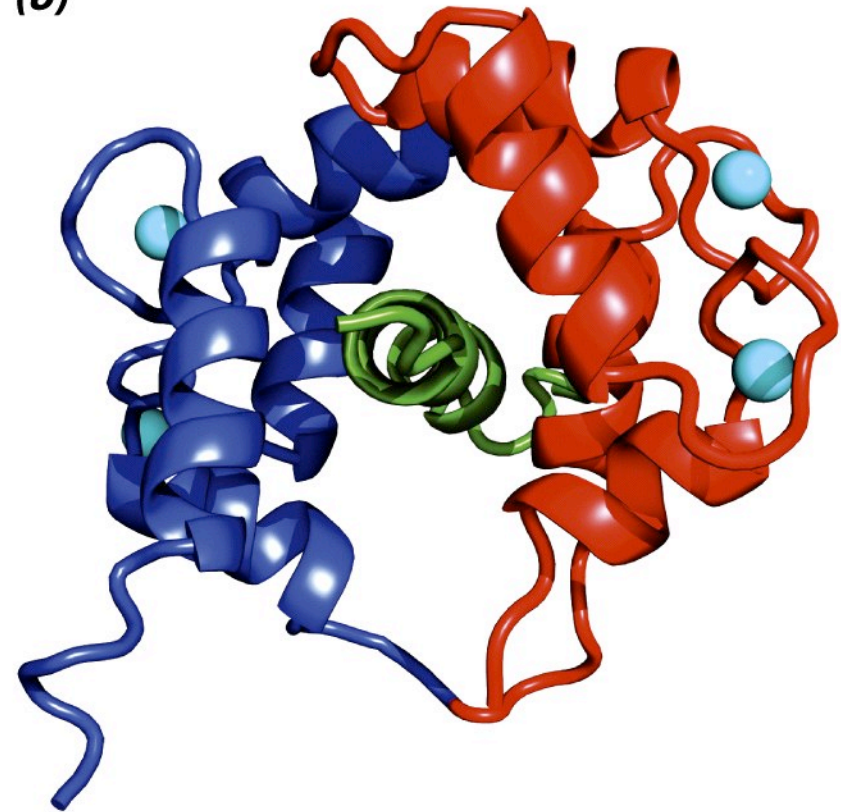


Domaine EF-hand

(a)



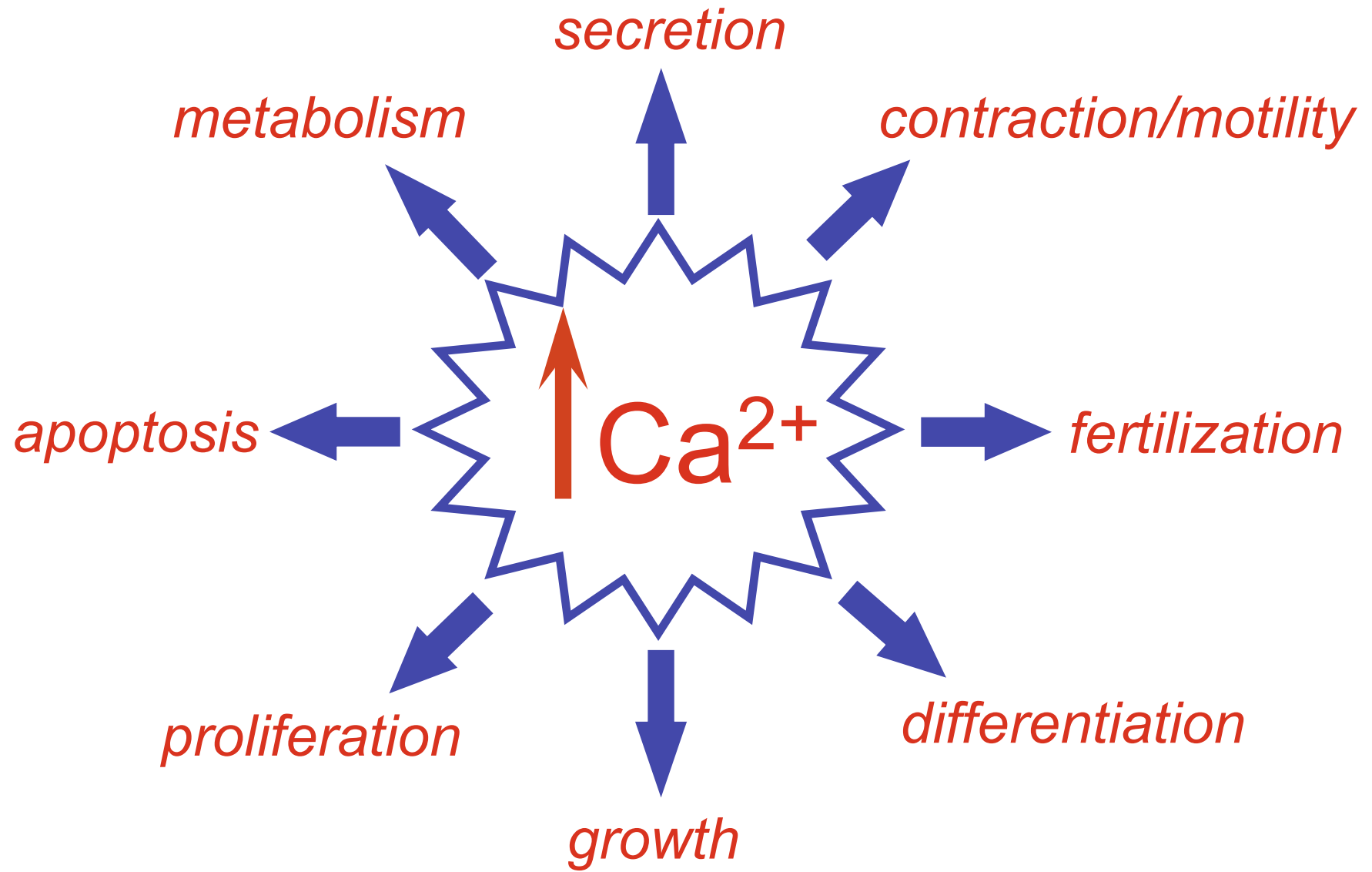
(b)



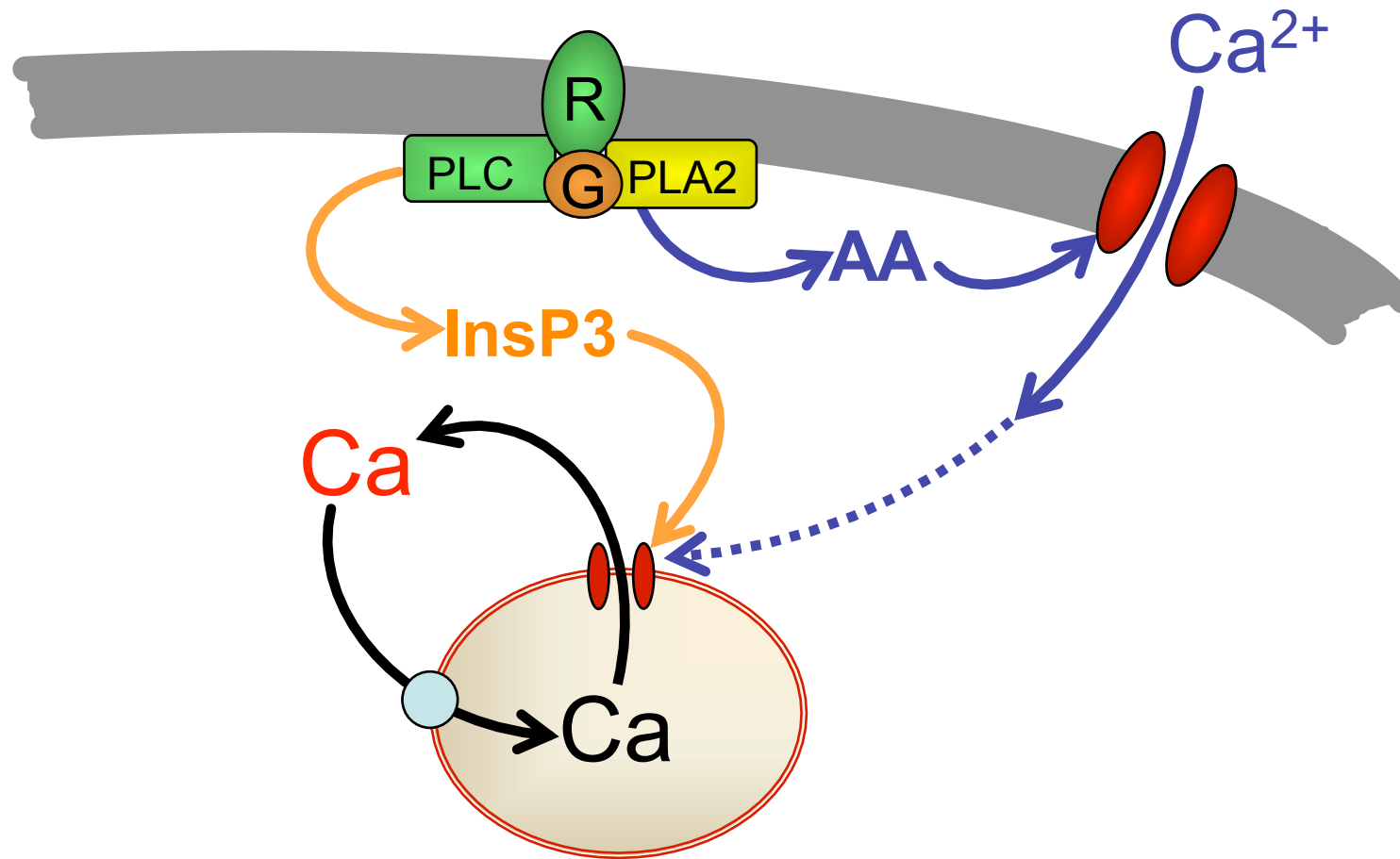
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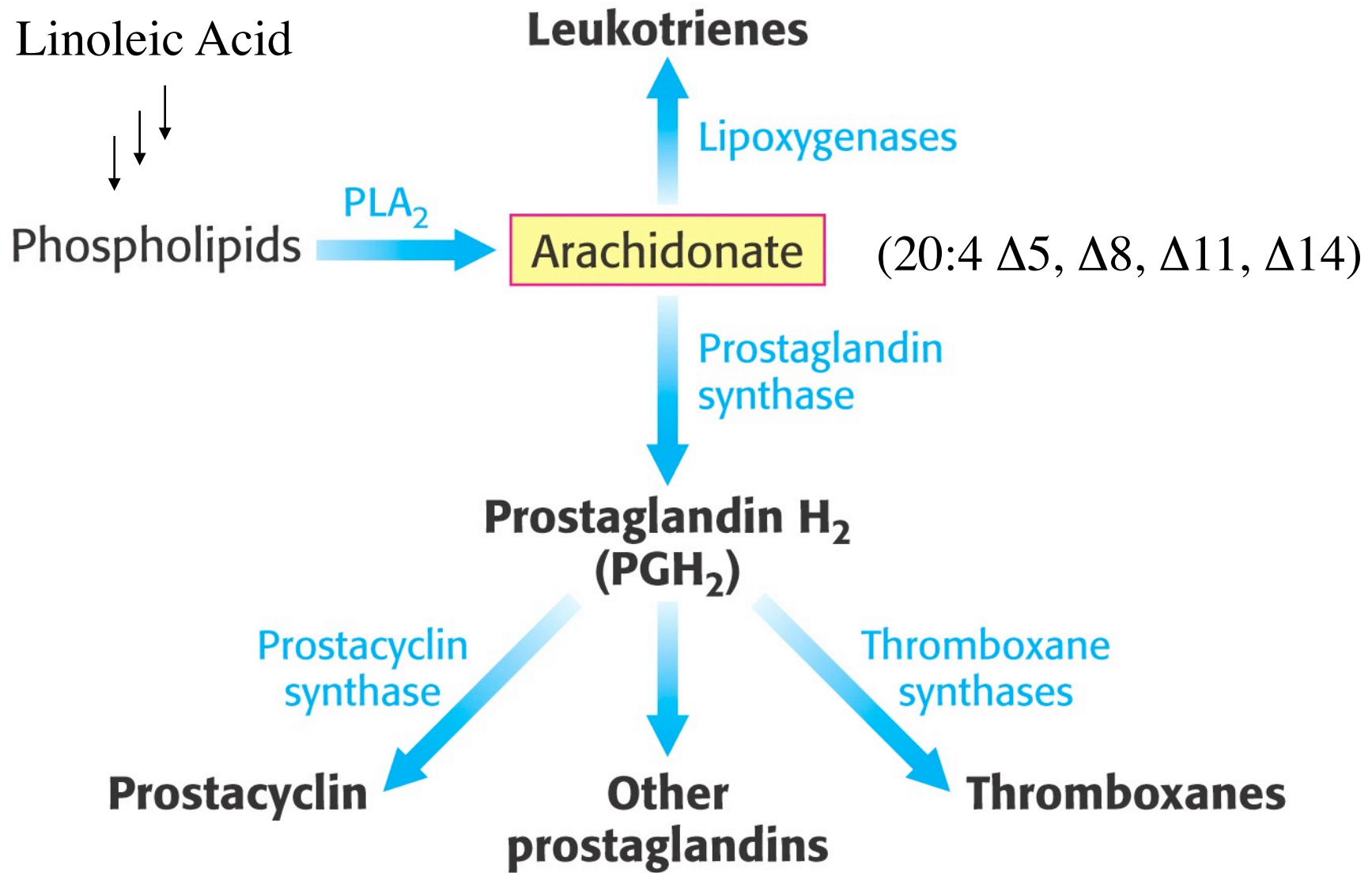
Calmoduline

Domaine EF-hand

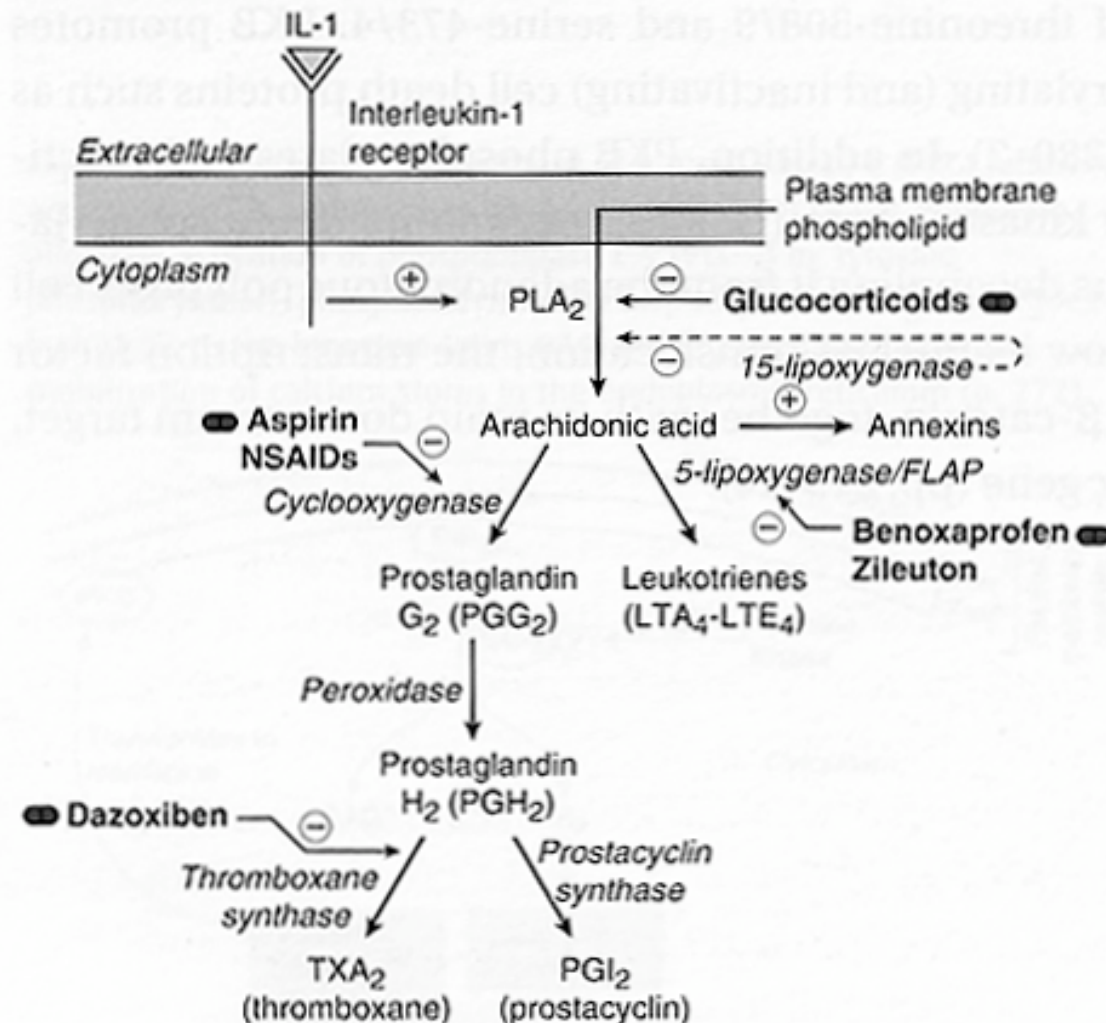


Ca^{2+} activates PLA_2 that triggers Arachidonic Acid Metabolism





Prostagandin biosynthesis pathways are a common drug target.



Interactions between G-proteins and RTKs

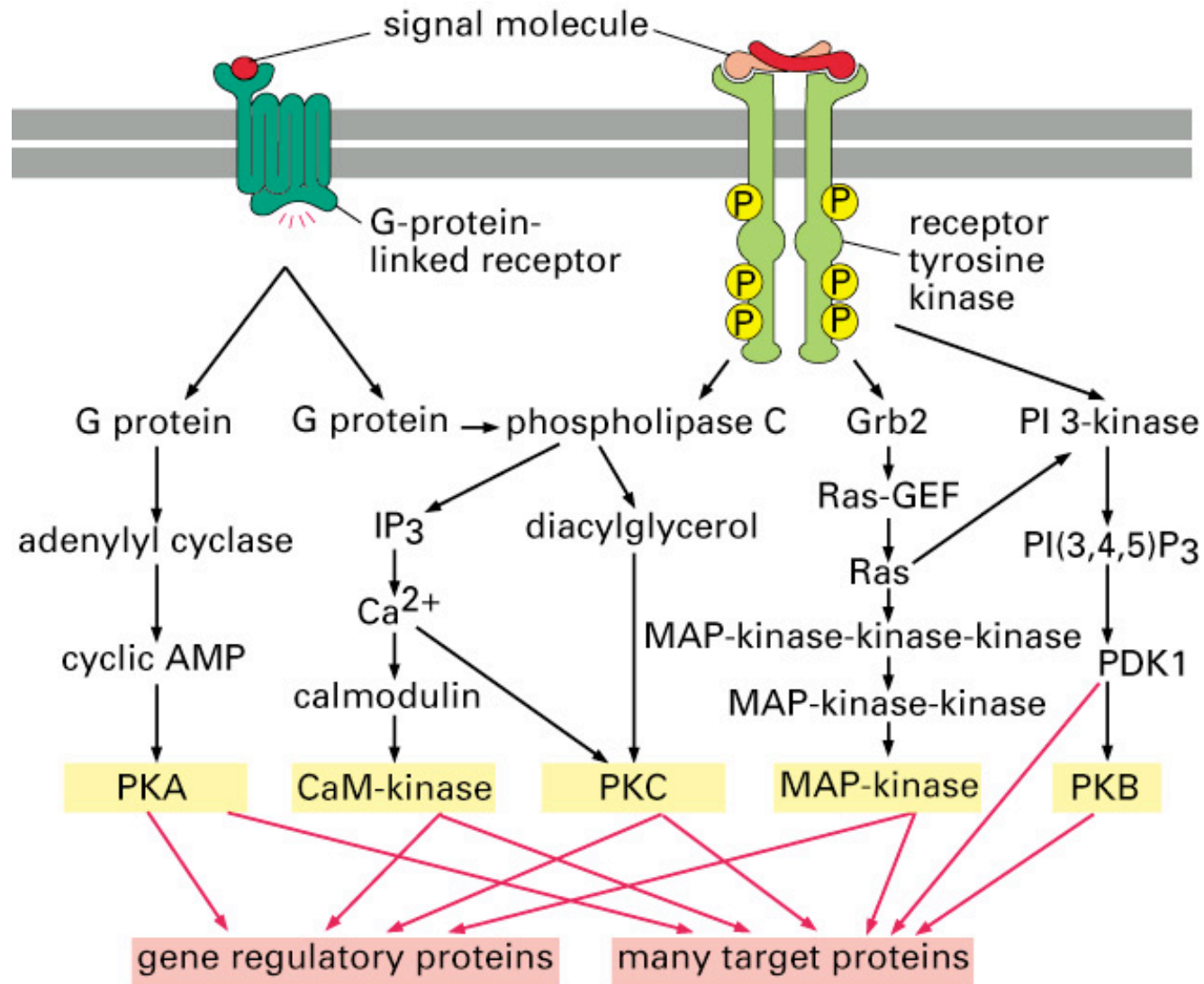


Figure 15-61. Molecular Biology of the Cell, 4th Edition.

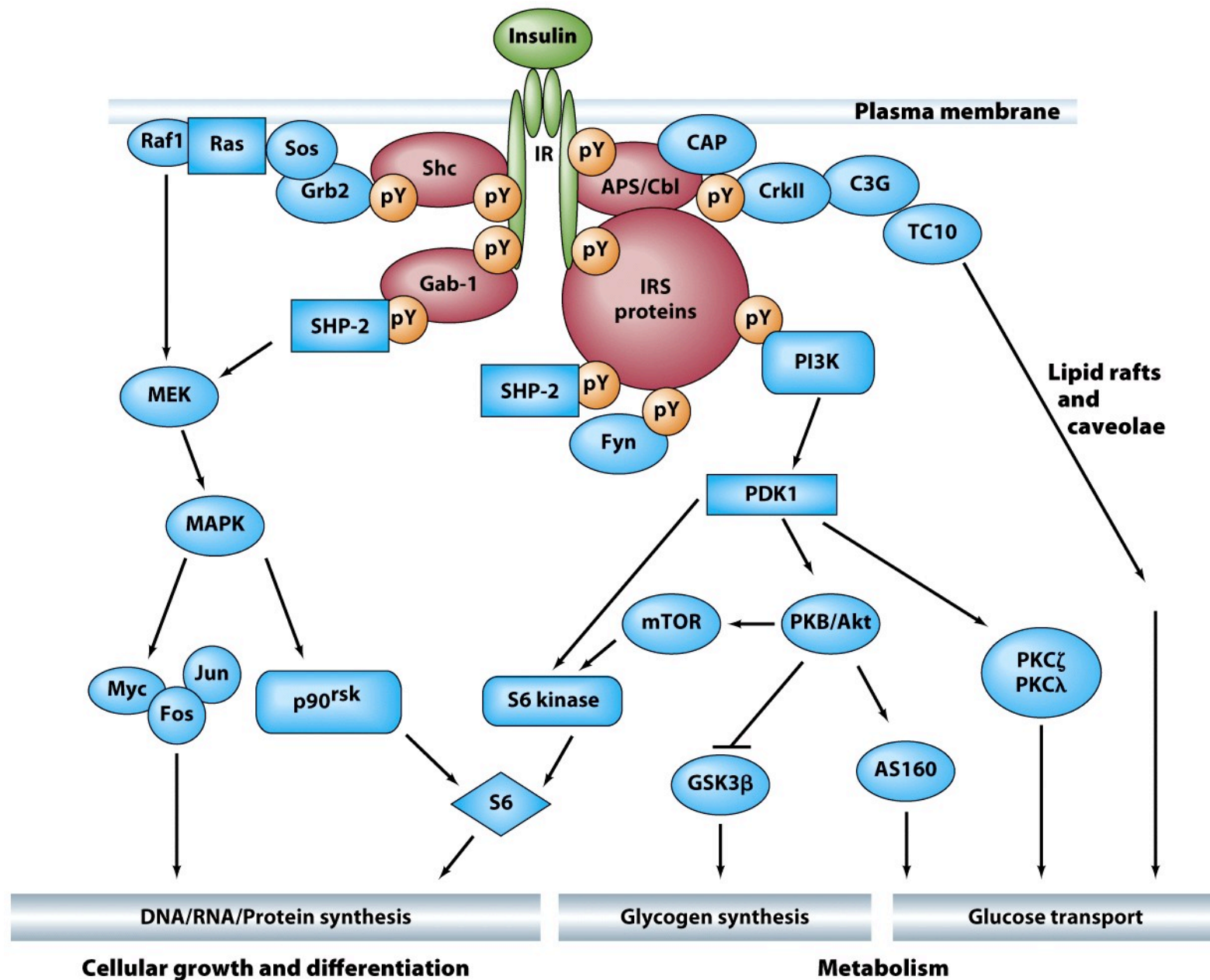
KINASES: RESUME

A/Ser-Thr kinases

–PKA: <i>cAMP dépendantes</i>	RRXSX
–PKC α,β,γ	XRXXSSRS
–PK-Ca-CAM-I	NYLRRLSDSNF
–PK-Ca-CAM-II	XRXXSX
–PK-Ca-CAM-III	RAGETRFTDTRK
–PKG: <i>cGMP dépendantes</i>	RXXSRX

B/ Tyr Kinases

–PKY lient –SH2/activent –SH3

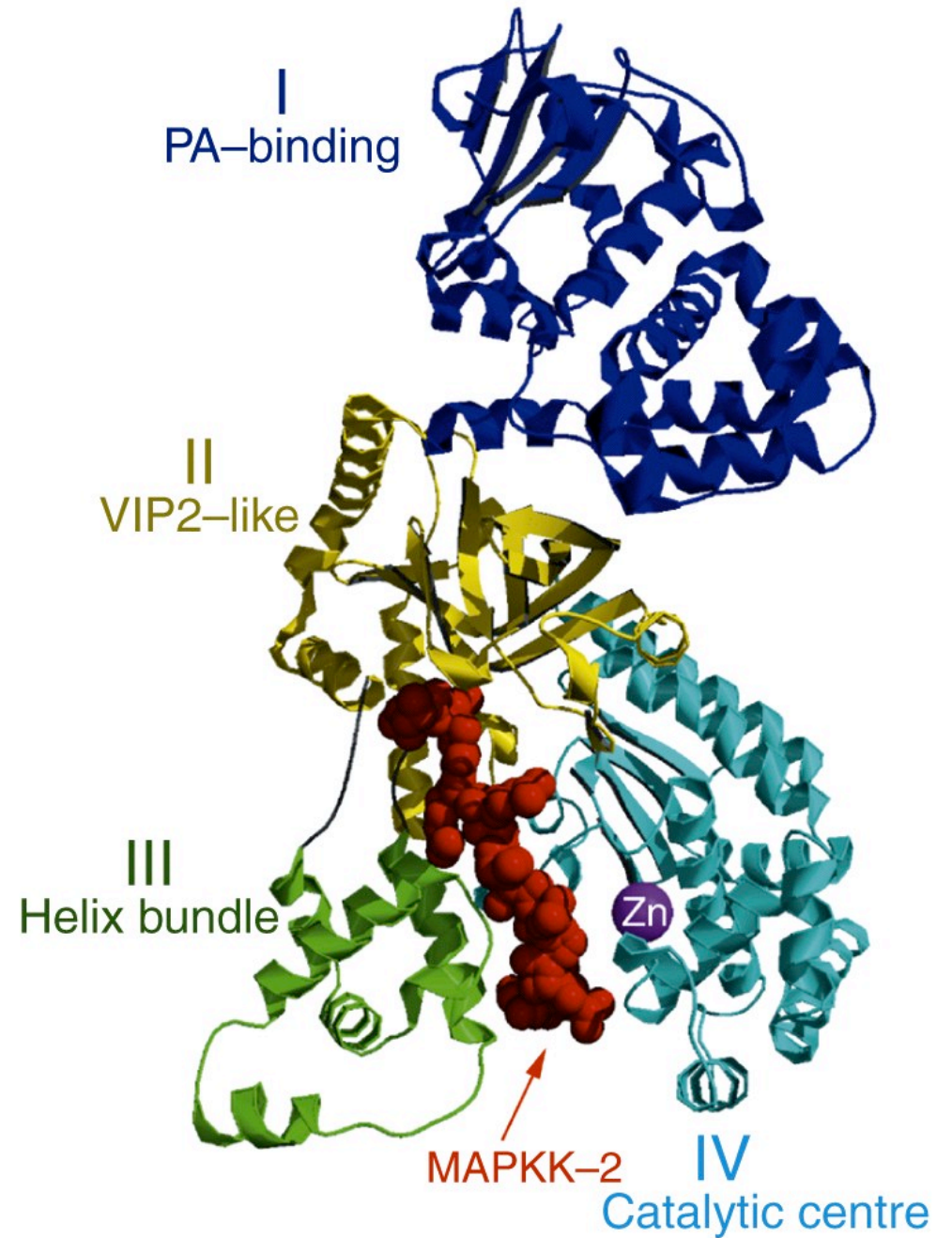


INSULIN SIGNAL TRANSDUCTION

Anthrax Spores



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Anthrax Lethal Factor